GENETIC DIFFERENTIATION, PHENOTYPIC PLASTICITY AND LATITUDINAL TRENDS IN NEW ZEALAND POPULATIONS OF *ERYTHRANTHE GUTTATA*

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Abstract

The New Zealand flora comprises proportionately more alien species than anywhere else on Earth. Many of these species elicit a variety of phenotypes across heterogenous environments and along a latitudinal gradient. Understanding what features lead to populations expressing multiple phenotypes is a key question in invasion biology. One hypothesis is genetic differences, which may be due to local adaptation, genetic drift, multiple introductions or a combination of these. Alternatively, phenotypic plasticity, which itself has a genetic basis, enables morphological and physiological alterations in response to changing environmental conditions. In New Zealand, the semi-aquatic herb *Erythranthe guttata*, 'monkey flower', is already showing signs of becoming invasive and is widespread across the South Island, blocking waterways and ditches. In this study I use common garden experiments to test for evidence of genetic differentiation, phenotypic plasticity and latitudinal trends in 35 populations of *E. guttata* from seven regions across the North and South Islands of New Zealand. My results have indicated significant genetic differences among New Zealand *E. guttata* populations and an ability to be phenotypically plastic which together is indicative of invasive potential. Furthermore, they highlight weak evidence for latitudinal trends among New Zealand populations. By replicating the common garden experiment for a second year I showed that maternal influences effect phenotype in *E. guttata* and that by reducing these influences I was able to provide stronger evidence for genetic diversity and clinal variation.

Chapter 1

Introduction

1.1 Invasion biology

Invasive species are of consequential importance to governments worldwide. They represent an ever-increasing threat to global economies and environments (Ellstrand & Schierenbeck, 2000; Hulme, 2012) and are now regarded as a significant driver of global change (Sakai *et al*., 2001; Lockwood *et al*., 2005; Proches *et al.*, 2008). Biological invasions ensue successful introduction, establishment and proliferation of a species in a novel environment (Facon *et al*., 2006). Modern-day invasions tend to occur as direct and indirect results of anthropogenic activities including land transformation, travel and trade (Vitousek *et al*., 1997; Sakai *et al*., 2001; Bossdorf *et al*. 2005; Waters & Grosser, 2016) and are becoming progressively more frequent. The mounting consequences of invasions include the detrimental erosion of native biodiversity (Vitousek *et al*., 1997; Facon *et al*., 2006) and severe economic impacts through both productivity losses and eradication schemes; estimates range from millions to billions of dollars per annum (Sakai *et al*., 2001; Hulme, 2012; The Royal Society of New Zealand, 2014).

1.1.1 Invasion process

The invasion process takes place in a series of key stages. The first stage of any invasion process is the introduction of an exotic species into a novel region (Sakai *et al*., 2001; Novak, 2007; Prentis *et al.*, 2008). This is followed by establishment, population expansion and further spread (Sakai *et al*., 2001; Lockwood *et al*., 2005; Prentis *et al.*, 2008).

1.1.2 Species introduction

For any species to inhabit an exotic range it first must be introduced. Introduction can be via accidental or intentional methods (Sakai *et al*., 2001). For example, hundreds of species of flora and fauna were introduced to New Zealand intentionally through European acclimatisation societies in the 1800 and 1900s (Wodzicki & Wright, 1984; Gravuer *et al.*, 2008; MacLeod *et al.*, 2009; Moles *et al.*, 2012). Conversely, many species have also been

introduced accidently such as through impure crop seed (Sakai *et al*., 2001) or on the tyre treads of imported second-hand cars. As a result of intentional and unintentional introductions, about 50% of the vascular flora in New Zealand is made up of nonindigenous species (Vitousek *et al.*, 1997; Proches *et al.*, 2008; Moles *et al.*, 2012).

1.1.3 Species establishment

While not well understood, exotics have been shown to undergo contemporary evolution when introduced to novel environments (Maron *et al*., 2004). Successful establishment of an organism may be determined by adaptive evolution among other possible mechanisms (Maron *et al.*, 2004). This ability to respond could be consequential of standing genetic variation or epigenetic variation (Prentis *et al.*, 2008).

Local adaptation is the evolution of a population of organisms to be better suited to their environment than conspecifics of other, geographically distinct, populations. The inherence to locally adapt depends on two components. The ability to react to microevolutionary processes dictated by the level of genetic diversity in phenotypic traits as well as the ability of individuals to express a diverse range of phenotypes in heterogenous environments (Michalski *et al.*, 2017). Commonly, adaptation is a phenotypic feature, shaped by prior evolutionary pressures and ultimately improves survival likelihood (Kawecki & Ebert, 2004). Ecological factors such as low gene flow, strong selection against previously optimal genotypes (genotypes adapted to the source environment), adaptive plasticity and, temporal variation in selection pressures are predicted to promote local adaptation (Kawecki & Ebert, 2004, Maron *et al*., 2004).

Genetic diversity governs a population's propensity to adapt to a novel or changing environment, thereby making it a critical factor in invasive potential (Handley *et al*., 2011). Traditionally, when a species is introduced to, or invades a new range, it is thought to undergo a genetic bottleneck (Prentis *et al.*, 2008; Liao *et al.*, 2016), however this is not always the case (Dlugosch & Parker, 2008). Loss of genetic diversity should limit a species potential for adaptive evolution (Prentis *et al.*, 2008) as well as perpetuating inbreeding (Ellstrand & Elam, 1993) relative to the source population (Sakai *et al*., 2001; Handley *et al*., 2011).

Mounting evidence highlights propagule pressure as an influential feature in invasion ability (Simberloff, 2009; Moles *et al.*, 2012; Miller *et al.*, 2015). Propagule pressure is the number of individuals released into a non-native location (Lockwood *et al*., 2005) and is observed through the interaction of propagule size (the number of individuals introduced) and propagule number (the pattern and number of introductions) (Lockwood *et al*., 2005; Simberloff, 2009). A large founding population will likely contain a large amount of genetic diversity buffering the population against environmental stochasticity (Lockwood *et al*., 2005, Miller *et al.*, 2015). Conversely, a large propagule number can ameliorate the effects of low genetic diversity through repeated introductions and increasing the likelihood of introducing new genetic diversity (Moles *et al.*, 2012; Hagenblad *et al.*, 2015).

1.1.4 Species expansion

Very few introduced species successfully establish (Novak, 2007; Hulme & Barrett, 2013) and fewer still become invasive (Ellstrand & Schierenbeck, 2000; Novak, 2007; Simberloff, 2009; Hulme, 2012). Of those that do become invasive, many undergo a long lag phase following the initial introduction and/or after multiple introductions (Ellstrand & Schierenbeck, 2000; Sakai *et al*., 2001; Lee, 2002; Bossdorf *et al*., 2005; Novak, 2007). The general rule of thumb is that roughly 10% of introduced species establish and of those that establish, a further 10% become invasive (Williamson, 1996). In New Zealand, the percentage of introduced species to have naturalised species is approximately 9% (Diez *et al.*, 2009; Hulme, 2012). A lag phase is thought to result from the need to garner sufficient levels of genetic diversity to overcome environmental invasive resistance as opposed to increasing population size (Lee, 2002). It has been suggested that rapid evolution of the invading species occurs during this lag phase, especially when the exotic species is exposed to ecogeographic variation (Prentis *et al.*, 2008; Stutz *et al.*, 2018). Rapid evolution may occur as a result of the invading species adapting to the new environment, hybridisation (Prentis *et al.*, 2008) and/or purging of genetic load (Hedrick, 1994; Ellstrand & Schierenbeck, 2000; Lee, 2002; Hodgins & Rieseberg, 2011; Colautti & Barrett, 2013; Matesanz *et al.*, 2015). Alternatively, the multiple introduction hypothesis suggests sufficient genetic diversity is obtained through intraspecific hybridization (Handley *et al*., 2011). However, it should be noted that studies have shown that genetic variation is not always necessary. In the case of *Impatiens glandulifera*, Hagenblad *et al.*,

(2015) were able to show that limited genetic diversity in their introduced range, compared to their native range, did not hinder it's invasive ability.

The final step in any invasion process is the expansion of the invading species into the new range (Sakai *et al*., 2001). Sustained range expansion is attributed to the adaptations of the exotic species. Several traits associated with the promotion of invasiveness have been identified, including the ability to reproduce both asexually and sexually, rapid growth, phenotypic plasticity and tolerance to environmental diversity (Sakai *et al*., 2001). It has been postulated that species possessing a culmination of these traits are far more likely to be invasive compared to species possessing only a few; however empirical data suggests this is not always the case (Sakai *et al*., 2001).

1.2 Theories for mechanisms of invasion

Plants can express hugely different morphologies and life history traits both within and between populations (Groot *et al*., 2017). These differences can be attributed to heterogeneity in the environment producing a variety of selection pressures (Groot *et al*., 2017). Introduction to a novel environment imposes vastly different selective pressures on an exotic species. To successfully establish and proliferate, the introduced species must overcome these selection pressures (Ebeling *et al*., 2011). Various theories have been proposed to understand and explain the invasive ability of species in novel habitats. A successful invasive species is suggested to possess at least one of three key characteristics; 1) physiologically matched to the novel environment (natural selection), 2) able to utilise sufficient phenotypic plasticity to maintain environmental fitness and/or, 3) have the ability to rapidly adapt to novel biotic and abiotic conditions (local adaptation) (Nicotra *et al*., 2010; Hulme & Barrett, 2013).

1.2.1 Natural selection and local adaptation

Charles Darwin was the first to posit the idea of natural selection. He describes natural selection as, "individuals best adapted to their environments are more likely to survive and reproduce." Theoretically populations have the potential to increase geometrically (Darwin, 1859) however in reality this doesn't happen. Darwin attributed this phenomenon to

competition between individuals for resources such as food (Darwin, 1859). Competition favours individuals who are better able to utilise limited resources, enabling them to survive and populate the next generation. Over subsequent generations, poorly adapted individuals (those lacking adaptations to maximise limited resources) fail to continue their genetic line, instead creating a population of well-adapted individuals. It is through this method that natural selection can lead to the evolution of populations and subsequently to the evolution of invasiveness.

Natural selection acts on pre-existing genetic variation and the accumulation of genetic differences within a population. The rate at which natural selection occurs is determined by the amount of additive genetic variation in a population. For example, a population with high additive genetic variation will be able to evolve superior and at a faster rate than a population with much lower levels of additive genetic variation (Nicotra *et al.*, 2010). Pre-existing adaptations can be indicative of invasive potential (Facon *et al*, 2006; Schlaepfer *et al*., 2010) and post-introduction adaptations perpetuate invasiveness (van Kleunen & Fischer, 2008). For example in plant species, it has been shown that populations displaying traits including tolerance to environmental heterogeneity, rapid growth and a large reproductive effort can be indicative of a pre-adaptation for invasive potential (Sakai, *et al*., 2001; Schlaepfer *et al*., 2010).

Local adaptation can be defined as the evolution of a population of organisms to be better-suited to the local environment than conspecifics from another population. This occurs through natural selection increasing the favourable trait frequencies which augments the survival and reproductive success of the population (Taylor, 1991; Ebeling *et al.*, 2011). It can be constricted by gene flow, and genetic drift and constrained from a lack of genetic variation in the population and frequent local extinctions (Kawecki & Ebert, 2004; Fraser *et al.*, 2011; Hamann *et al*., 2017). Local adaptation has been posited as being responsible for genetic variation in morphological, behavioural, physiological and biochemical among and between populations (Taylor, 1991). It can be observed both on a broad geographic scale and microgeographically (Taylor, 1991; Ebeling *et al*., 2011; Fraser *et al.*, 2011; Vergeer & Kunin, 2013). Consideration of local adaptation can be important in conservation prioritization or in the development of restoration programmes (Fraser *et al.*, 2011).

1.2.2 Phenotypic plasticity

Phenotypic plasticity has historically been regarded as a primary driver enabling the rapid adaptation of a population in a novel environment (Maron *et al.*, 2004; Liao *et al.*, 2016) by overcoming the time period required for genetic adaptations to evolve (Michalski *et al.*, 2017; Munzbergova *et al.*, 2017). Phenotypic plasticity has a genetic basis whereby a single genotype has the ability to express multiple phenotypes (Pigliucci *et al*., 1995) and can respond to changing selective pressures (Michalski *et al.*, 2017; Munzbergova *et al.*, 2017). It enables an individual to alter the expression of various physiological, morphological, behavioural and demographic features in response to changes in environmental conditions (Miner *et al*., 2005). Plastic responses can occur across generations or be expressed within the lifespan of a single individual (Miner *et al.*, 2005) assisting rapid adaptation (Nicotra *et al.*, 2010).

Phenotypic plasticity is thought to facilitate the invasion process as it broadens an individual's environmental niche and therefore, its potential habitat range (Hulme & Barrett, 2013). Essentially it creates a 'general-purpose' genotype that alters phenotypic expression enabling the introduced species to survive and persist in heterogenous environmental conditions where local adaptation has not yet occurred (Bossdorf *et al.*, 2005; Hulme, 2008; Palacio-Lopez *et al.*, 2015; Liao *et al.*, 2016; Hamann *et al.*, 2017). Plasticity would provide a fitness advantage to an invading species suffering from a lack of genetic variation which would prevent adaptation through natural selection (Bossdorf *et al.*, 2005).

1.3 Experimental designs

Two common methods have been used when studying the life histories of plants; reciprocal transplants and common gardens (Sexton *et al.*, 2009; Fraser *et al.*, 2011). Reciprocal transplants involve introducing individuals originating from different environments into the original habitats of one another (Kawecki & Ebert, 2004). Alternatively, the common garden method involves re-creating the essential facets of the different habitats into one common environment and transplanting individuals from the different environments into it (Kawecki & Ebert, 2004). Both methods are often used to determine the presence of underlying genetic

components potentially responsible for observable differences in phenology (Hulme & Barrett, 2013) as well as directly testing the role of environmental factors in driving divergent selection and local adaptation (Kawecki & Ebert, 2004).

1.3.1 Common garden

Common gardens are a prevalent method for exploited for exploring the evolution within invasive species. Classically, they have been used to determine whether observable differences among populations of a species are genetically based or result from phenotypic plasticity (Hufford & Mazer, 2003; Hirano *et al*., 2017). They have been used to test geographic range limits (Angert *et al.*, 2008; Dixon & Busch, 2017), fixation of evolutionary novel genotypes, fitness benefits due to heterosis (Ellstrand & Schierenbeck, 2000), local adaptation (Kawecki & Ebert, 2004; Hall & Willis, 2006; Peterson *et al.*, 2016), latitudinal trends in phenology (Kollmann & Banuelos, 2004) as well providing an insight into the speed or scale of local adaptation (Fraser *et al.*, 2011; Stutz *et al.*, 2018). A common garden recreates the essential properties of different habitats while controlling for other factors (Kawecki & Ebert, 2004). This can occur in the laboratory, glasshouse or in experimental plots (Kawecki & Ebert, 2004). Unlike in reciprocal transplants, environmental factors can be more or less controlled and standardized between conspecifics thereby minimising confounding factors. These factors include, but are not limited to, differences in sunlight radiation, sunlight hours, precipitation, shadings and wind.

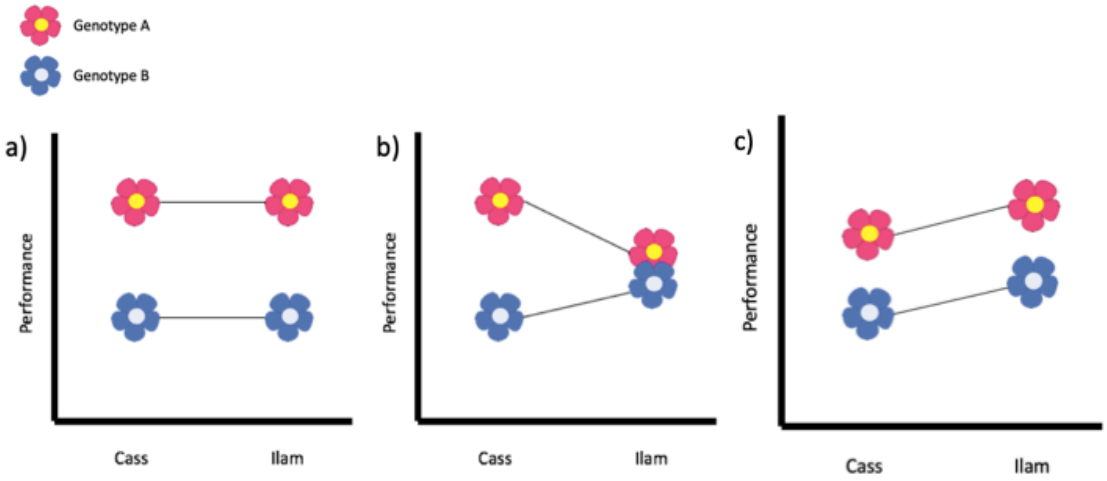

Figure 1. 1: Delineating between genetic differentiation, genotype by environment (GxE) interaction and phenotypic plasticity. Two common gardens can differentiate between what are genetic differences and phenotypic plasticity. On this diagram garden locations, 'Cass' and 'Ilam' are on the x-axis, while performance is measured on the y-axis. The blue flower represents one genotype and the pink flower represents a second genotype. Each common garden experiences a distinctly different environment. a) shows a pattern of genetic differentiation whereby genotype A and genotype B perform the same at the two common garden sites; b) shows a GxE interaction (and phenotypic plasticity) whereby genotype A and genotype B differ in terms of their performance at the two common gardens. Genotype A decreases its performance from Cass to Ilam and genotype B increases its performance; c) shows a pattern of just phenotypic plasticity whereby genotype A and genotype B increase by the same amount at both common garden sites.

A conceptual issue with common garden experiments is that they cannot differentiate between divergence resulting from genetic drift or natural selection (Hufford & Mazer, 2003; Ebeling *et al.*, 2011). The most effective practice to overcome this is to perform multiple common garden experiments in different environments (Figure 1.1) (Nuismer & Gandon, 2008; Ebeling *et al.*, 2011). The multiple common garden approach directly tests the interaction between genotypes and the environment (GxE). While not tested in this study, the multiple garden approach can be used to test for local adaptation (Kawecki & Ebert, 2004; Nuismer & Gandon, 2008; Cheplick, 2015). It minimises the contribution of environmental

variation and as such isolates the genetic variation attributed to fitness (Nuismer & Gandon, 2008; Ebeling *et al*., 2011).

1.4 Summary

Understanding the source of phenotypic variation in invasive species is important; whether differences among populations are genetic or plastic has major implications for control. Untangling the source of variation – i.e. whether it is genetic or plastic can be achieved using common garden experiments.

1.5 Study species, *Erythranthe guttata*

Figure 1. 2: Erythranthe guttata

1.5.1 Biology and introduction to New Zealand

Erythranthe guttata (Figure 1.2), (Fisch. ex DC.) G.L. Nesom, formerly known as *Mimulus guttatus* (Barker *et al.*, 2012), family Phrymaceae, is a semi-aquatic herb species native to western North America (van Kleunen *et al.*, 2015). Introduced for ornamental and horticultural purposes in the late 1800s (Webb *et al*., 1988), *E. guttata* has naturalised in New Zealand as a weed species (Champion & Clayton, 2001). It occurs predominantly in riparian habitats, from lowland to montane regions throughout the country, however it is present in a wide range of environments, and seems tolerant to those which vary in temperature, shade, soil type and wind enabling it to persist in all regions of New Zealand (Grant, 1924; Hall & Willis, 2006).

The first herbarium specimen of *E. guttata* in New Zealand is from Whanganui, Manawatu-Whanganui recorded in the 1940s (Landcare Research Manaaki Whenua, 2018). *E. guttata* can now be found throughout New Zealand from as North as Whangarei to as far South as Invercargill (Landcare Research Manaaki Whenua, 2018).

Erythranthe guttata is a fast-growing herbaceous species growing to approximately 60cm tall and 60cm wide (Northland Regional Council, 2017), although some individuals can grow in excess of one metre tall (Truscott *et al.*, 2008). The plants produce several oval, bright green leaves with serrated edges which vary in degrees of hairness. Characteristically, they produce bright yellow tubular flowers with small, red dots within the corolla. In New Zealand, they flower between November to March. Flowering is followed by production of numerous small fruits containing containing hundreds of thousands of seeds.

The Department of Conservation has listed *E. guttata* as being naturalised, uncontrolled but not banned from sale (Champion & Clayton, 2001). The Northland Regional Council is the only New Zealand council at present to actively remove *E. guttata* from waterways. Once introduced into a region, *E. guttata* rapidly overtakes and outcompetes native macrophytes. It's aggressive vegetative reproduction and long growing season (Kiang & Hamrick, 1978) dominate and eclipse native species which typically form shallow mats and lack competitive ability (Collins *et al.*, 2018).

1.5.2 Reproduction and spread

In its native range of North America, *Erythranthe guttata* occurs in one of two main ecotypes; annual and perennial (Murren & Dudash, 2012; van Kleunen *et al.*, 2015; Peterson *et al*., 2016). Annual populations have a life cycle lasting for one calendar year and tend to inhabit areas with cyclic wet and dry seasons (Lowry *et al*., 2008; van Kleunen *et al.*, 2015). Perennial populations have a life cycle extending beyond one calendar year and are often found in areas which remain wet year-round (Grant, 1924; Lowry *et al*., 2008; van Kleunen *et al.*, 2015). Primarily *E. guttata* is a perennial species but populations will default to annual growth when exposed to substandard growing conditions (Kiang & Hamrick, 1978).

Erythranthe guttata reproduces sexually through the production of tiny, windborne seeds which are usually outcrossed but may be selfed (Willis, 1993; van Kleunen & Fischer, 2008; Murren *et al.*, 2009; van Kleunen *et al.*, 2015). Novel environments tend to impose restrictions on sexual reproduction such as fewer potential mates, and low pollination rates. To overcome such restraints, plants have evolved to propagate through the spread of vegetative fragments in water (Kiang & Hamrick, 1978; Truscott *et al.*, 2006; Barrett *et al*., 2008; Murren *et al.*,

2009; van Kleunen *et al.*, 2015; Collins *et al.*, 2018). Vegetative reproduction has been noted to aid the successful capabilities of *E. guttata* during the early stages of an invasion (Trtikova *et al.*, 2011). People are also able to spread *E. guttata*; seeds are easily stuck on the soles of shoes and fragments get moved in soil (Gravuer *et al.*, 2008; Collins *et al.*, 2018).

Of note is that in Scotland, where it is also introduced, *E. guttata* has recently undergone an autopolyploidization event (Simon-Porcar *et al*., 2017). Genetic analyses have shown that this population of autopolyploids have evolved from a local diploid population in the Shetland Islands within the last 200 years. This neo-autotetrapolyploid population show marked differences in morphology and phenology including larger, more robust individuals which are less inclined to flower compared to diploid individuals (Simon-Porcar *et al*., 2017). In its native range *E. guttata* will actively hybridise with related species such as *Mimulus luteus* (Vallejo-Marin & Lye, 2013) and *E. nasutus* (Kiang & Hamrick, 1978). While some hybrids are sterile, they are generally capable of vegetative growth and become established in riparian habitats (Kiang & Hamrick, 1978; Vallejo-Marin & Lye, 2013).

1.5.3 Threat to native biota

The New Zealand flora comprises proportionately more exotic species than anywhere else on Earth (Hulme, 2018). More than 20% of New Zealand's flora is considered to be under threat from invasive species and 86% of these threatened species are indigenous (Dopson *et al*., 1999). Many of these species were introduced by acclimatisation societies to 'enrich' the New Zealand biota (Wodzicki & Wright, 1984; MacLeod *et al.*, 2009; Moles *et al.*, 2012). *E. guttata* was one of these species and was introduced for its ornamental properties and use in horticulture (van Kleunen & Fischer, 2008). As such it has been introduced serveral times (van Kleunen & Fischer, 2008). The multiple introductions would suggest that most of the genetic variation expressed by *E. guttata* in its native range has now been introduced to New Zealand (van Kleunen & Fischer, 2008) which theoretically should give it great invasive potential (Prentis *et al.*, 2008).

Erythranthe guttata has the potential to change the ecology of riparian systems. The riparian zone is identified as the strip of vegetation along the banks of rivers, lakes, streams and wetlands (Collins *et al.*, 2013). Over the past 30 years, riparian restoration has been

undertaken in New Zealand (Collins *et al.*, 2013). These regions have been recognised as being highly susceptible to invasion due to their increased propensity for disturbance and high resource availability (Miller *et al.*, 2015). They also act as source habitats for the further spread of invasive species (Miller *et al.*, 2015). *E. guttata* has been shown to decrease biodiversity in invaded with experiments demonstrating that invaded sites show a decreasing species richness with increasing percentage cover of *E. guttata* (Truscott *et al*., 2008). It's excessive growth can negatively affect waterways through the reduction of water flow, chokage of drainage systems, increased sediment deposition and ultimately altering the aquatic community structure (Collins *et al.*, 2018).

1.6 The study sites, Christchurch and Cass

I chose two study sites for my two common gardens, varying significantly in climate and altitude.

1.6.1 University of Canterbury, Christchurch

Figure 1. 3: University of Canterbury, Christchurch.

My main common garden was chosen for logistical reasons. It is in the grounds of the University of Canterbury, Christchurch (Figure 1.3) (43°31'S 172°35'E). The elevation is 15 m about sea level. Christchurch has a mean annual rainfall of 131.3 mm. It has a relatively dry climate with summer temperatures that average around the mid to high-twenties (December-February) and winter temperatures that average at around 3°C (Climate & Weather Averages in Christchurch, New Zealand, 2018; NIWA, 2018a). Throughout this thesis, the University of Canterbury common garden will be referred to as 'Ilam'.

1.6.2 University of Canterbury, Cass Field Station

Figure 1. 4: University of Canterbury, Cass Field Station.

My second common garden was at the University of Canterbury Cass Field Station (Figure 1.4), located in the Selwyn District of the Canterbury region (43°02'S, 171°45'E) at 577 m above sea level. *Erythranthe guttata* occurs naturally at Cass, growing in the nearby stream. The field station consists of 1775 ha of eastern South Island mountain land. It compromises two prominent hills, Cass Hill (1098 m) and Sugarloaf Hill (1359m) as well as river terraces and alluvial fans. The Cass Basin provide a wide range of environments – montane grasslands, scrub, riverbed, scree, beech forest, swamp, bog, lake, stream and alpine habitats. Rain is frequent with a mean annual rainfall of 2289.6 mm. In the summer months, the temperature can supersede 20°C and during winter months will frequently go below 0°C (NIWA, 2018b). Temperatures at Cass fluctuate far more than the temperatures in Christchurch. Throughout this thesis, the Cass Field Station common garden will be referred to as 'Cass'.

1.7 The aim and overall structure of this thesis

1.7.1 Overall aim

This study has three main aims:

1) To identify whether phenotypic differences observed in New Zealand populations of the exotic herb *Erythranthe guttata* are due to genetic differentiation or phenotypic plasticity.
2) To determine whether plant performance traits varied along a latitudinal gradient, indicative of the evolution of a latitudinal cline.
3) The final aim is to determine the influence of maternal effects on plant performance.

Understanding the adaptive ability of this species is crucial for developing control systems and the eventual eradication of this exotic species.

1.7.2 Thesis outline

The remainder of the thesis has been split into four major sections. The following three sections are dedicated to the experimental components of the research; genetic differentiation and phenotypic plasticity, latitudinal trends and maternal effects. The final section will be an assembly of all of the results in a general discussion of the research, a suggestion of future directions and the significance of the results to the management of this invasive weed, *Erythranthe guttata*.

Each chapter does not completely stand alone as I have tried to remove repetitive content. However, there is repetition of ideas and information throughout this thesis.

Figure 1. 5: The Ilam common garden (Photo credit: C. Antony).

Figure 1. 6: The Cass common garden (Photo credit: I. Williamson).

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Chapter 2

Untangling the sources of phenotypic variation in New Zealand populations of *Erythranthe guttata*

2.1 Introduction

Across New Zealand, in a wide variety of habitats (Chapter 1) *Erythranthe guttata* displays a plethora of phenotypes. The cause of this phenotypic differentiation may be plastic i.e. the same genotype producing a different phenotype in different environments. Alternatively, or in addition to, the phenotypes may differ genetically. The reason for genetic differences among populations may be due to local adaptation, genetic drift, multiple introductions or a combination of these (Bossdorf *et al.*, 2005; Cheplick, 2015).

Natural selection acts on pre-existing genetic variation within a population. It favours the survival of phenotypic variants that are best suited to the local environment (Darwin, 1859; Latta, 2010). Over generations, natural selection can lead to an increase in advantageous-heritable traits which improve lifetime fitness of individuals relative to those lacking the traits (Antonovics, 1976; Latta, 2010; Cheplick, 2015). A genotype by environment (GxE) interaction may be defined as a change in the relative trait performance of two or more genotypes when measured in two or more disparate environments (see Figure 1.1). The interactions may involve reorganization of the rank order of genotypes between environments and equate to phenotypic variances between environments (Bowman, 1972; Schlichting, 1986). Consequentially, GxE interactions create divergent selection pressures favouring different genotypes in different environments and establishing local adaptations (Cheplick, 2015). Local adaptations to select habitats are common among species that occupy a broad spatial scale (Vergeer & Kunin, 2012; Hamann *et al.*, 2017) and result in ecotypic specialization. It has been observed in a number of studies and summarized by Schlichting (1986), that clones grown in different environments change trait expression, highlighting an effect of this relationship reorganization. Pre-existing adaptations may facilitate invasive success (Facon *et*

al, 2006; Schlaepfer *et al*., 2010), while post-introductory adaptations may perpetuate invasive ability (van Kleunen & Fischer, 2008). If post-introduction natural selection is responsible for *E. guttata* adaptation in New Zealand habitats, then different populations should be genetically distinct and local adaptation would be evident. As such, we would typically expect locally adapted populations to exhibit a home-site advantage when establishing in foreign habitats (Montalvo & Ellstrand, 2000). A home-site advantage generally dictates that individuals will perform best in environments which closely match those to which they are adapted (Cheplick, 2015). Genetic differentiation can result in plant populations from different habitats eliciting distinctly different phenotypes.

While ecotypic specialization presents a fitness advantage relative to other genotypes in the local environment, not all genotypes show local adaptation. Instead, the genetic trait of phenotypic plasticity can encourage establishment into new environments (Sexton *et al.*, 2002; Bennington *et al.*, 2012). Phenotypic plasticity is an evolvable, genetic trait which allows a single genotype to express multiple 'optimal' phenotypes when raised in heterogenous environments (Pigliucci *et al*., 1995; DeWitt *et al*., 1998; Sultan, 2000; Agrawal, 2001; Pigliucci, 2007; Palacio-Lopez *et al.*, 2015; Liao *et al.*, 2016). This phenomenon does not require genetic variation to produce the myriad of phenotypes. Instead a single, 'general-purpose gentoype' facilitates the ability to express multiple phenotypes (Bossdorf *et al.*, 2005; Hulme, 2008) and enables an organism to thereby respond to a novel environment within a single generation, and maintain maximal fitness in unfavourable conditions (Schlichting, 1986; Sultan, 2004; Richards *et al.*, 2006).

Phenotypes which respond to environmental differences can include changes in morphology, physiology, life-history and behaviour (Sultan, 2004; Miner *et al.*, 2005). Phenotypic plasticity is commonly observed in founding individuals providing the population with the ability to reside and flourish in heterogenous new environments (Richards *et al.*, 2006). This could be especially important because founding populations may suffer from a lack of genetic variation, preventing adaptation via natural selection (Liao *et al.*, 2016). In turn, phenotypic plasticity could promote future adaptive evolution (Liao *et al*., 2016). Unlike genetic differentiation, plastic responses can occur at the individual level; within the lifespan of an individual, or across generations (Miner *et al.*, 2005). Phenotypic plasticity is thought to be extremely important in the invasion process where the native species are more specialised

and at the pinnacle of their evolutionary ability (DeWitt *et al*., 1998; Sultan, 2003; Hulme, 2008).

Several key traits have been identified has being under strong selection in introduced populations. Traditionally, it was thought that that particular traits may predispose a population (or species) to becoming invasive, however modern research has identified that the same traits are shared by successful, noninvasive species (Thompson & Davis, 2011; Kuester *et al.*, 2014; Bock *et al.*, 2015) or are idiosyncratic to individual species (Moles *et al.*, 2012). Any trait has the ability to evolve invasive capabilities, including life-history, phenological (Kollmann & Banuelos, 2004; van Kleunen & Fischer, 2008; Allan & Pannell, 2009; Bull-Herenu & Arroyo, 2009; Vitasse *et al.*, 2009), morphological and physiological traits (Li *et al*., 1998; Kollmann & Banuelos, 2004; van Kleunen & Fischer, 2008; Weijschede *et al.*, 2008; Allan & Pannell, 2009; Lowry *et al.*, 2012; Michalski *et al*., 2017; Munzbergova *et al.*, 2017). Anthocyanins are a less studied performance measure but are an effective trait for studying the evolution of phenotypic variation (Lowry *et al.*, 2012). Responsible for pigmentations in plants (Chalker-Scott, 1999; Lowry *et al.*, 2012), anthocyanins can be indicative of environmental stress or cellular damage (Chalker-Scott, 1999; Picotte *et al.*, 2007) caused by strong light, UV exposure, temperature, precipitation and pollutants (Merzlyak & Chivkunova, 2000; Lowry *et al.*, 2012).

2.1.1 Phenotypic differences among populations of *Erythranthe guttata*

Elsewhere, numerous studies have highlighted phenotypic differences among populations of *Erythranthe guttata* in heterogenous environments. For example, differences have been revealed in flowering time (Hall & Willis, 2006), number of flowers (Lowry *et al.*, 2008), floral characteristics (Murren *et al.*, 2009; Murren & Dudash, 2012), vegetative vs reproductive growth (Peterson *et al.*, 2016), leaf characteristics (Lowry *et al.*, 2008) and anthocyanin concentration (Lowry *et al.*, 2012). Virtually all studies on *E. guttata* have been conducted on populations in North America and the United Kingdom and very few have been conducted on New Zealand populations (van Kleunen & Fischer, 2008; Collins *et al.*, 2018). New Zealand studies such as van Kleunen and Fischer (2008) and Collins *et al.* (2018) have assessed plant performance and management techniques. In the present study I assessed plant performance by five measures:

1. Plant size (by measuring the above ground dry weight, average largest leaf length and width, longest horizontal and vertical shoot length and internode length).
2. Reproductive output (maximum number of flowers and maximum number of buds).
3. Stress (by scoring anthocyanin colouration).
4. Phenology (Julian date of first bud, first flower, maximum bud number and maximum flower number).
5. Floral characteristics (by measuring the largest flower height, depth and width).

There are two main explanations for the occurrence of variable phenotypes across divergent habitats; populations from different habitats are genetically different from one another, or this species is able to respond plastically to its surrounding environment. It is also likely that the explanation is a combination of both genetic differentiation and phenotypic plasticity (Pigliucci, 2007; Liao *et al.*, 2016).

2.1.2 Objective One

The first objective of my research is to determine whether the sampled populations of *Erythranthe guttata* maintain their trait differences in a common garden, indicative of genetic differences among them. Conversely, if no obvious genetic differences are found across habitats and regions when all plants are grown together under the same environment, the successes of the species may be a consequence of phenotypic plasticity.

2.2 Methods

2.2.1 Experimental Background

A classic method for investigating whether phenotypic differences observed in the field are the result of phenotypic plasticity or local adaptation is the implementation of common garden experiments (Olsson & Agren, 2002). This method involves sampling populations from multiple different habitats in the field and translocating them into a garden where the environmental factors can be more or less controlled. Each individual will experience as identical growing conditions (e.g. light, water, nutrients, and temperature) as possible.

2.2.2 Population locations and sampling

In order to collect *Erythranthe guttata* populations from a representative sample of climatic and latitudinal conditions across New Zealand, I first looked at NIWA climatic summaries (NIWA, 2017) which include data from 1971-2000. From these data, I designed a sampling. I identified populations of *E. guttata* from seven geographically-separate regions within New Zealand; North Island (NI), South Island Central (SI_C), South Island Central East (SI_CE), South Island North East (SI_NE), South Island North West (SI_NW), South Island South East (SI_SE), South Island South West (SI_SW). The details of each region in terms of key climatic variables are presented in Table 2.1. Within each region, I identified several populations – the exact number varied across regions and depended on what I observed from the roadside. In total, 35 populations were collected (Table 2.2). These 35 populations defined a variety of habitats including those which varied in temperature, latitude, rainfall, sunlight hours, and altitude (Figure 2.1 and Table 2.1). Within each population, I collected a handful of ramets from a single clonal individual (Figure 2.2d).

Populations were found in a variety of locations such as on roadsides, drainage ditches and along rivers and streams (Figures 2.2a and 2.2b). At each population, I sampled what I thought to be one vegetative clone. From this clone either six or 12 replicates were produced from shoot tip or internode cuttings (Figure 2.3). The number of replicates made from each clone depended on whether or not I was going to use the population in both Ilam and Cass common gardens. I made 12 cuttings for populations grown at both common garden sites, and six for populations grown only at the Ilam common garden. Extra plants were propagated for each population to account for individuals which did not successfully propagate and to fill remaining spaces in the Ilam common garden. In total, I produced 366 individuals from 35 populations (clones) (Figure 2.2). I first planted the propagated individuals into slow-release fertilizer on January 1, 2018 and kept them in a University of Canterbury glasshouse for two weeks to establish roots before I transferred them into larger pots outside. Plants were grown in either the Ilam or the Cass common garden over the summer months of 2017-2018.

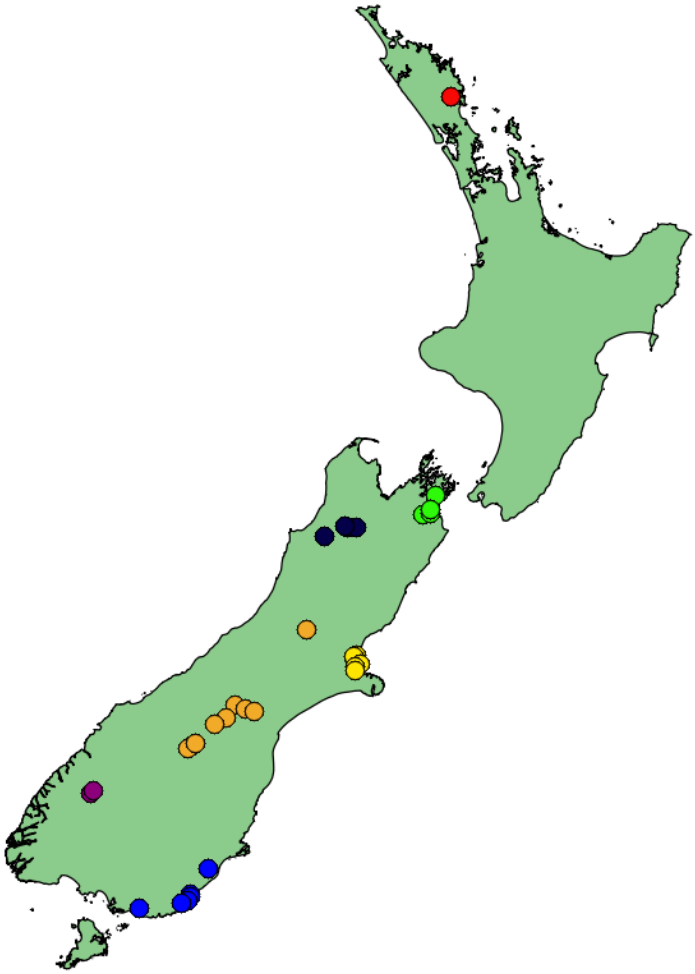

Figure 2. 1: A map of New Zealand indicating the sampling locations for each of the 35 populations. The geographically-distinct regions have been colour coded; North Island (NI) = red, South Island Central (SI_C) = orange, South Island Central East = yellow South Island North East (SI_NE) = green, South Island North West (SI_NW) = navy blue, South Island South East (SI_SE) = blue, South Island South West (SI_SW) = purple. The map was produced using QGIS (QGIS Development Team, 2018).

Table 2. 1: Sampling locations and reasons for each location. These decisions are all based on NIWA climatic summaries (NIWA, 2017). Locations are positioned in either the North Island (NI) or South Island (SI) of New Zealand. Locations have been divided into seven regions; North Island (NI), South Island Central (SI_C), South Island Central East (SI_CE), South Island North East (SI_NE), South Island North West (SI_NW), South Island South East (SI_SE), South Island South West (SI_SW).

NI vs SI	Region	District/City	Reason
NI	NI	Whangarei	Most northern latitude
SI	SI_C	Mackenzie District	Average coolest location and highest altitude (Tekapo)
SI	SI_CE	Christchurch and North Canterbury	Lowest altitude
SI	SI_NE	Marlborough	Average warmest location, lowest rainfall and highest number of hours of sunlight (Blenheim)
SI	SI_NW	Tasman	Moderate environmental conditions
SI	SI_SE	Fiordland	Highest annual rainfall
SI	SI_SW	Otago	Most southern location and fewest number of hours of sunlight

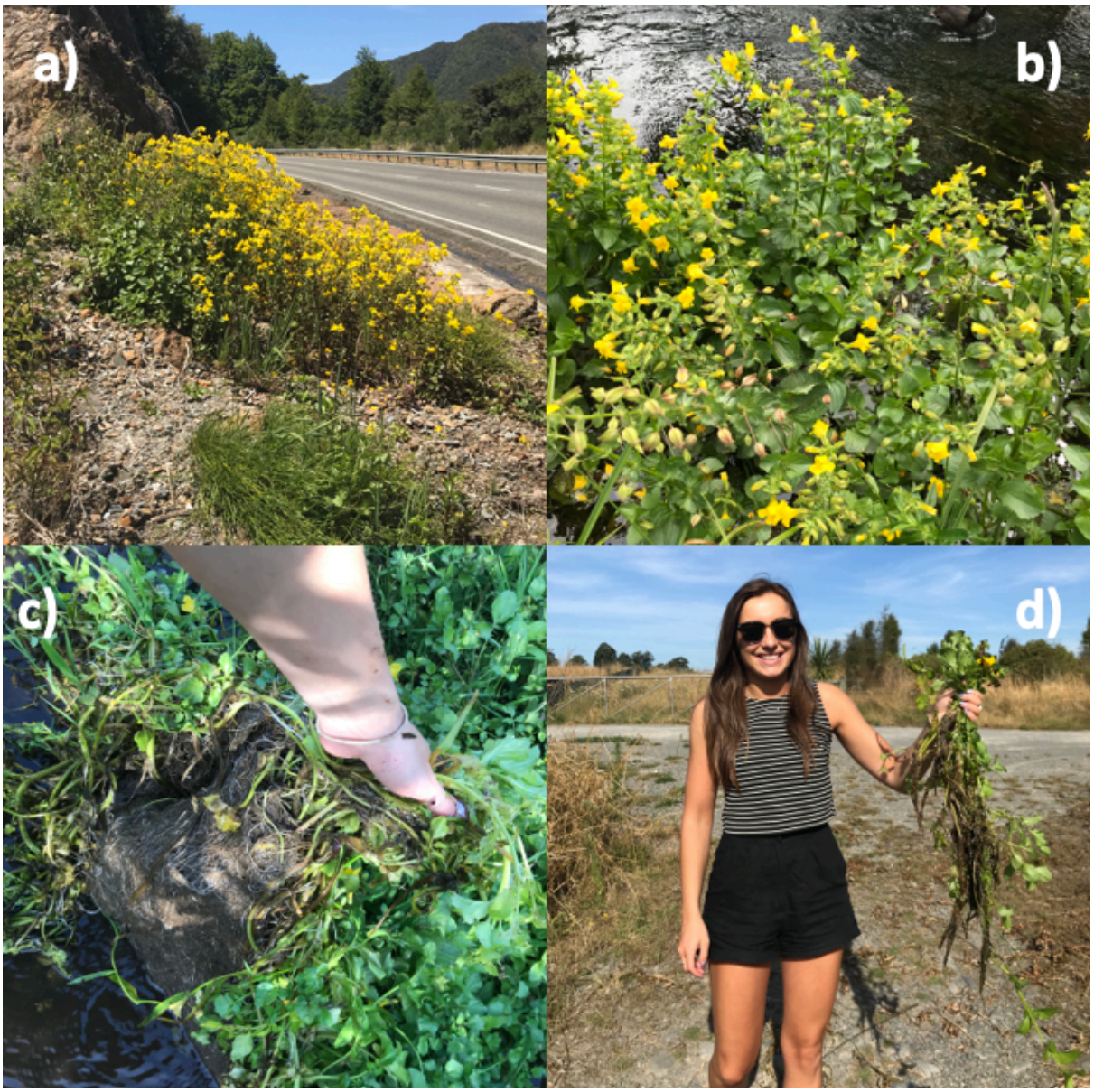

Figure 2. 2: Erythranthe guttata *in the field; a) cluster of roadside* E. guttata *in Kahurangi National Park (SI_NW); b) cluster of* E. guttata *growing along the riverbank of the Avon River, Christchurch (SI_CE); c) the rhizomatous growth of* E. guttata*; d) collecting a cluster of* E. guttata *ramets (Photo credit: I. Williamson).*

Figure 2. 3: A series of tip cuttings (above) and internode cuttings (below) of Erythranthe guttata.

I recorded 16 environmental variables for each sampling location (Table 2.2). I calculated the annual average temperature, growing season average temperature, average minimum and maximum temperature, extreme minimum and maximum temperature, annual average rainfall, humidity and precipitation from weather data collected at local weather stations (Table 2.3) (CliFlo, 2018). The inconsistent distance between weather station and population location may cause bias in the results and therefore I took this into consideration during analysis and in the result interpretation of results.

*Table 2. 2: The environmental variables recorded or measured for each of the 35 populations across New Zealand. Temperatures were measured in "℃", altitude measured in "m", rainfall and precipitation in "mm". *0 means no, 1 means yes. **Environmental averages for the growing period (September – March).*

Region	Location Name	Population	Latitude	Longitude	Water Logged*	Flowing Water*	Altitude (m)	Annual Average Temperature	Average Temperature**	Average Min Temperature**	Extreme Min Temperature**	Average Max Temperature**	Extreme Max Temperature**	Annual Average Rainfall	Humidity**	Precipitation**	Temperature at Collection	Sunlight Hours	n llam	n Cass
SI NW	Kawatiri-Murchison Highway	1	-41.695	172.478	0	0	271	12.36	15.16	8.26	-1.55	22.08	32.85	871.6	74.4	70.6	25	15.03	9	0
SI NW	St Arnaud-Kawatiri Highway	2	-41.697	172.637	0	1	271	9.88	12.48	6.13	-5.7	18.83	30	871.6	74.4	70.6	24	15.03	8	0
SI NE	Taylor River, Blenheim 1	3	-41.509	173.954	1	1	7.55	13.33	15.53	10.26	0.17	20.79	31.64	433.9	78.7	30.9	28	15.01	6	5
SI NE	Hawkesbury Road, Hawkesbury	4	-41.524	173.817	1	1	59.25	12.68	14.98	8.88	-1.94	21.04	32.69	433.9	78.7	30.9	28	15.01	7	5
SI NE	Taylor River, Blenheim 2	5	-41.512	173.960	1	1	8.6	13.33	15.53	10.26	0.17	20.79	31.64	433.9	78.7	30.9	28	15.01	6	6
SI NE	Waikawa Road, Waikawa	6	-41.273	174.033	1	1	18.56	13.54	15.51	11.64	2.3	19.4	27.61	433.9	78.7	30.9	28	15.01	9	7
SI NE	Rapaura Road, Spring Creek	7	-41.459	173.959	1	1	8.23	13.33	15.53	10.26	0.17	20.79	31.64	433.9	78.7	30.9	17	15.01	12	3
SI NE	Hillocks Road, Spring Creek	8	-41.457	173.950	1	1	8.77	13.33	15.53	10.26	0.17	20.79	31.64	433.9	78.7	30.9	17	15.01	10	5
SI NW	Kahurangi National Park	9	-41.685	172.441	0	1	250.63	12.36	15.16	8.26	-1.55	22.08	32.85	871.6	74.4	70.6	24	15.01	8	3
SI NW	Upper Buller Gorge	10	-41.811	172.064	0	1	204.4	12.36	15.16	8.26	-1.55	22.08	32.85	871.6	74.4	70.6	24	15.01	9	4
SI C	Fairlie-Tekapo Road, Lake Tekapo	11	-44.007	170.488	1	1	714.63	9.01	12.01	3.09	-4.7	18.53	30.86	493.1	76.9	40.5	26	15.25	6	0
SI C	Tekapo-Twizel Road, Tekapo	12	-44.175	170.323	1	1	519.71	9.82	13.24	5.35	-5.6	21.13	33.15	493.1	76.9	40.5	26	15.24	6	0
SI C	Tekapo Twizel Track, Pukaki	13	-44.254	170.116	1	1	457.22	9.82	13.24	5.35	-5.6	21.13	33.15	493.1	76.9	40.5	26	15.24	8	0

SI C	Omarama-Lindis Pass Road, Waitaki 6	14	-44.505	169.781	1	1	576.41	9.82	13.05	6.1	-4.2	19.99	33.03	493.1	76.9	40.5	26	15.27	6	0
SI C	Omarama-Linids Pass Road, Waitaki 2	15	-44.532	169.712	1	1	664.48	9.82	13.05	6.1	-4.2	19.99	33.03	493.1	76.9	40.5	26	15.27	6	0
SI C	Omarama-Lindis Pass Road, Waitaki 10	16	-44.565	169.662	1	1	664.48	9.82	13.05	6.1	-4.2	19.99	33.03	493.1	76.9	40.5	26	15.27	8	0
SI C	Omarama-Lindis Pass Road, Waitaki 4	17	-44.506	169.782	1	1	664.48	9.82	13.05	6.1	-4.2	19.99	33.03	493.1	76.9	40.5	26	15.27	8	0
SI C	Fairlie-Tekapo Road, Mackenzie	18	-44.066	170.672	0	0	501.14	10.6	12.71	6.82	-1.98	17.04	31.03	493.1	76.9	40.5	26	15.25	8	0
SI C	Geraldine-Fairlie Highway	19	-44.097	170.834	1	0	298.31	10.6	12.71	6.82	-1.98	17.04	31.03	493.1	76.9	40.5	26	15.25	6	0
SI SE	Waihola Highway, Milburn	21	-46.074	170.013	1	1	44.53	10.37	12.69	6.57	-3.56	18.3	31.81	1080.5	79.2	92.9	16	15.43	9	6
SI SE	Owaka Highway, Clutha	22	-46.377	169.690	1	0	60	9.99	12.03	7.02	-2.08	17.03	28.53	1080.5	79.2	92.9	12	15.47	6	1
SI SE	Owaka Highway, Katea, Otago	23	-46.419	169.693	0	0	99.03	10.33	11.95	8.33	0.78	15.58	27.56	1080.5	79.2	92.9	12	15.47	10	0
SI SE	Papatowai Highway, Owaka	24	-46.459	169.646	0	0	75	10.33	11.95	8.33	0.78	15.58	27.56	1080.5	79.2	92.9	16	15.47	10	6
SI SE	Papatowai Highway, Clutha	25	-46.487	169.545	1	0	177.44	10.35	11.96	7.39	-0.13	16.56	29.04	1080.5	79.2	92.9	16	15.47	6	5
SI SE	Tokanui-Gorge Road Highway, Fortrose	26	-46.560	168.790	0	0	4.26	10.77	12.41	8.78	0.24	15.39	19.85	1080.5	79.2	92.9	26	14.46	6	4
SI SW	Te Anau-Milford Highway, Southland	27	-45.137	167.931	0	1	339.92	9.29	11.66	5.25	-3.26	16.47	28.32	644.8	66.3	54.2	23	15.38	6	6
SI CE	Styx Mill Reserve, Northwood	29	-43.464	172.609	1	1	11.92	11.64	14.12	8.71	-2.66	19.53	33.12	131.3	75.3	10.1	25	15.25	6	5
SI CE	Keating Street, Silverstream	30	-43.379	172.634	1	1	6.44	11.67	13.92	8.4	-1.22	19.44	32.7	131.3	75.3	10.1	25	15.25	7	3
SI CE	Jeffs Drain Road, Ohoka	31	-43.394	172.598	1	1	10.14	11.67	13.92	8.4	-1.22	19.44	32.7	131.3	75.3	10.1	25	15.25	8	6
SI CE	Travis Wetlands Heritage Park	32	-43.485	172.699	0	1	2.05	12.29	14.7	9.63	-0.62	19.76	32.5	131.3	75.3	10.1	25	15.25	8	4
SI CE	Park Terrace, Avon River	33	-43.523	172.627	1	1	9.69	12.29	14.7	9.63	-0.62	19.76	32.5	131.3	75.3	10.1	25	15.25	8	6
SI CE	Ashgrove Terrace, Heathcote River	34	-43.567	172.627	1	1	10.22	12.29	14.7	9.63	-0.62	19.76	32.5	131.3	75.3	10.1	25	15.25	8	0
NI	The Quarry Arts Centre, Whangarei	35	-35.722	174.311	0	1	38.24	15.97	17.61	14.14	4.64	22.44	29.23	492.7	78	35.2	24	14.35	8	4

SI C	Cass Field Station	36	-43.035	171.758	1	1	578.81	8.22	10.72	4.17	-5.3	17.27	30.06	2289.6	84	192.6	19	14.45	6	6
SI SW	Fiordland National Park, Fiordland	37	-45.101	167.968	0	1	279.77	8.22	10.72	5.25	-3.26	16.47	28.32	644.8	66.3	54.2	23	15.38	8	0

Table 2. 3: Weather station information for each of the 35 populations. The weather station closest to the population location was selected and the distance between the population location and weather station recorded.

Population	Station Number	Distance (km)
1	16826	17.6
2	31850	20.9
3	12430	1.3
4	4326	3.9
5	12430	1.5
6	4232	19.8
7	12430	4.4
8	12430	4.8
9	16826	16.5
10	16826	21.5
11	24945	3.6
12	36596	17.6
13	36596	2.3
14	5212	8.9
15	5212	14.1
16	5212	18.5
17	5212	8.8
18	37255	8.4
19	37255	8.3
21	7339	21.6
22	26163	9.9
23	5893	9.5
24	5893	12.6
25	5904	12.6
26	5823	31.7
27	37382	18.9
29	4843	6.6
30	17244	5.9
31	17244	7.3
32	4858	8.2
33	4858	1.1
34	4858	4.1
35	40980	2.9
36	4651	13.6
37	37382	14.2

2.2.3 Common garden set up

A 10m x 10m section of land at the University of Canterbury was gifted for use during the duration of this research. I lined the land with black weed matting to inhibit unwanted weed growth. I planted the cuttings of *Erythranthe guttata* into individual pots (7.5L, 205mm high x 255mm diameter) that I had lined with plastic bags and filled with a slow release fertilizer potting mix. An automated watering system ensured the common garden was watered thoroughly once a day at 7:15am for 10 minutes using a Hunter 4-station irrigation controller watering system. I used a randomized design to arrange the 252 pots into a series of rows (Figure 2.4).

Figure 2. 4: The Ilam garden layout on January 26, 2018. This image was taken from a drone (Photo credit: C. Antony).

I set up a second common garden experiment at the University of Canterbury's Cass Field Station. A 20m x 3m section was blocked off and covered with black weed mat for use. I used

the same pots and watering system at Cass as I used at Ilam. As with Ilam, I used a randomized design to arrange the pots. I arranged the Cass garden into three rows (Figure 2.5) which in total contained 100 pots. It was logistically difficult to have two large gardens so I produced the Cass garden as a subset of the populations grown in the Ilam garden (Table 2.2).

Figure 2. 5: The Cass garden layout on January 17, 2018.

The use of two gardens that experience different environmental conditions is crucial when studying phenotypic plasticity and genetic differences (Williams *et al.*, 2008). Two common gardens provide far stronger evidence than a single garden (Cheplick, 2015) and can differentiate between genetic differences and phenotypic plasticity (see Figure 1.1). The varied environmental features between gardens enables the assessment of population performance across diverse conditions (Cheplick, 2015). Genetic differences between

populations would see the same phenotype expressed in both gardens as the genotype has been selected for over a series of generations. However, if the populations are expressing phenotypic plasticity, the phenotype may change so as to be optimal for the environment in which it is raised in. The Ilam and Cass common gardens experience different environmental conditions creating different selective pressures for plant survival and reproduction (Table 2.4).

*Table 2. 4: Environmental measures of Ilam, Christchurch and the Cass Field Station. Temperatures were measured in "℃", altitude measured in "m", rainfall and precipitation measured in "mm". **Environmental averages for the growing period (September – March).*

Region	Location	Altitude	Annual Average Temperature	Average Temperature**	Average Min Temperature**	Extreme Min Temperature**	Average Max Temperature**	Extreme Max Temperature**	Annual Average Rainfall	Humidity**	Precipitation**	Max Sunlight Hours	Min Sunlight Hours
SI CE	Ilam, Christchurch	9.69	12.29	14.7	9.63	-0.6	19.76	32.5	131.3	75.3	10.10	15.25	8.27
SI C	Cass Field Station	578.81	8.22	10.72	4.17	-5.3	17.27	30.06	2289.6	84	192.6	15.22	9

2.2.4 Performance measurements

To determine whether local adaptation has occurred in New Zealand populations of *Erythranthe guttata*, the plant performance of each individual was recorded over time. Table 2.5 outlines the plant performance measures for this experiment:

Table 2. 5: The plant performance traits measured at Ilam and Cass over the summer of 2017-2018.

Performance Measure	Unit of Measurement
Above ground dry weight	g
Average largest leaf length (Figure 2.6)	mm
Average largest leaf width (Figure 2.6)	mm
Longest horizontal shoot	mm
Longest vertical shoot	mm
Internode length (measured between the second and third internodes from the tip (Figure 2.7b))	mm
Anthocyanin score (0 = none, 1 = low, 2 = moderate, 3 = high)	0-3
Date of first bud	Julian date
Date max of bud	Julian date
Max bud number (Figure 2.6)	
Largest flower height (Figure 2.6)	mm
Largest flower depth (Figure 2.6)	mm
Largest flower width (Figure 2.6)	mm

I chose these performance measures because numerous studies on *E. guttata* and similar species in both their native and invasive ranges have already shown variation among these phenotypic and morphologic traits, both in the field and in common garden experiments (Chalker-Scott, 1999; Kollmann & Banuelos, 2004; Lowry *et al*., 2008; Weijschede *et al.*, 2008; Murren *et al*., 2009; Ebeling *et al*., 2011; Frei *et al*., 2012; Murren & Dudash, 2012; Vergeer & Kunin, 2013; Hamann *et al*., 2017; Groot *et al*., 2018). Biomass, leaf measurements and shoot lengths are commonly used as a relative measure of plant size (Groot *et al*., 2018). The leaf and shoot lengths are a commonly used non-destructive method of quantifying biomass. Anthocyanin concentration is indicative of plant stress levels (Chalker-Scott, 1999). Flower measurements have been previously used by Murren *et al*. (2009) to assess differences in trait means under common environmental conditions. The remaining performance traits (maximum bud number, date of first bud and date of the maximum bud number) give an estimate of the relative reproductive output of each individual (Murren *et al*., 2009; Ebeling *et al*., 2011).

For analysis purposes, I used the maximum value recorded for each individual over the entire study period was used for flower height, depth, width and bud number. Leaf length and width were the average of the two largest leaves prior to harvesting. I collected all other morphological measures in the week prior to harvesting. I collected floral measures and bud numbers every two or three days between January 20 until April 1, 2018. I measured the initial plant sizes by weighing the tip and internode cuttings to determine any variation in starting size that may affect the final growth performance results.

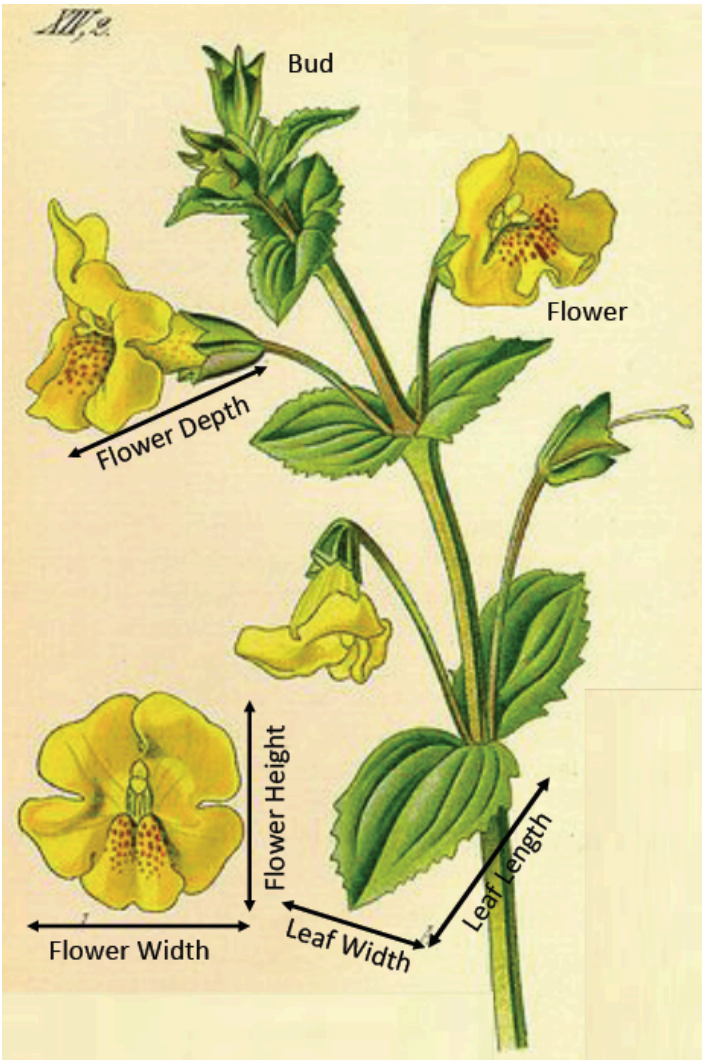

Figure 2. 6: Diagram of Erythranthe guttata *illustrating how seven of the performance traits were measured. Adapted from Pinterest (2018).*

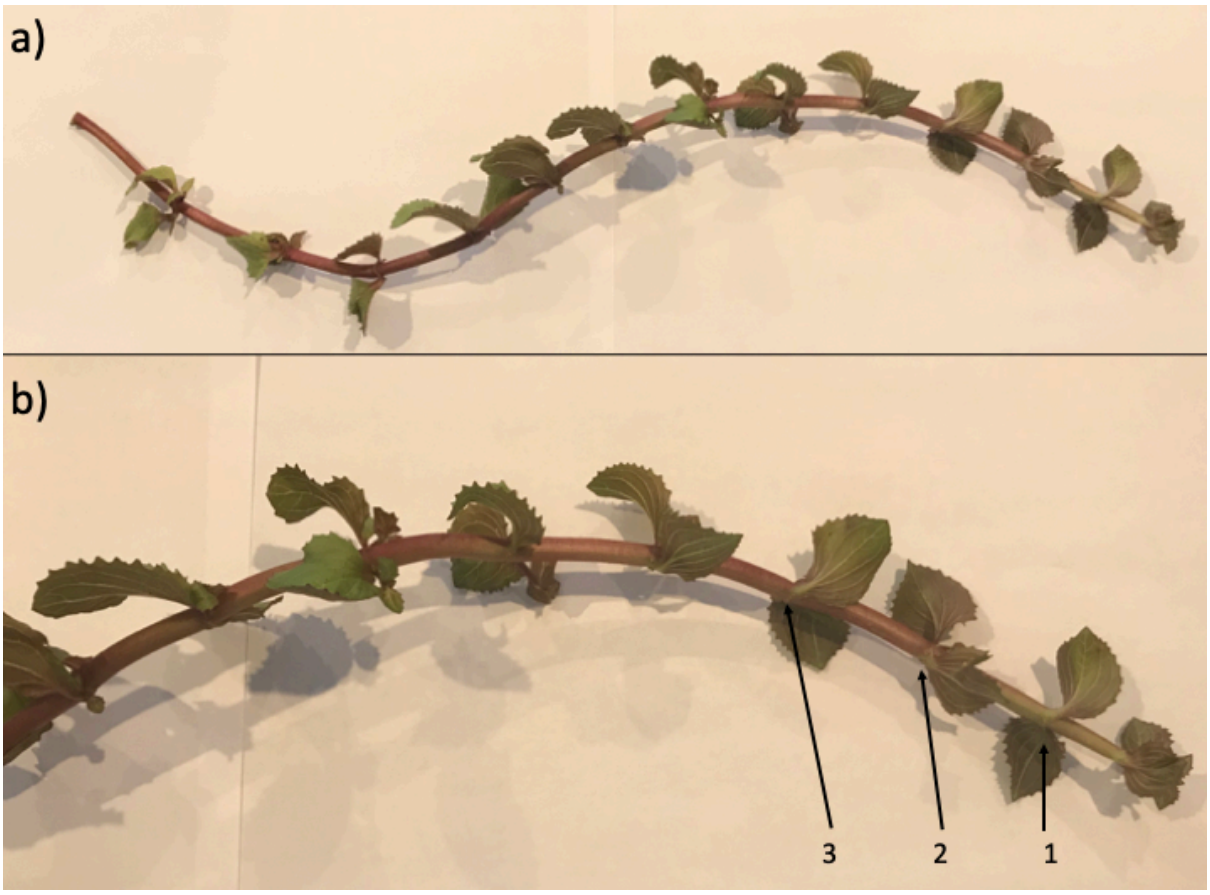

Figure 2. 7: a) the longest shoot on an Erythranthe guttata *plant. b) the end of a shoot depicting the first '1', second '2' and third '3' internodes from tip.*

2.2.5 Analysis

In the methods, I explained that at each site (population) I collected one plant. As plants tend to spread through vegetative growth (Figures 2.2a to 2.2c) rather than a single stem, I collected a bunch of closely intertwined stems (Figures 2.2c and 2.2d). I assumed these were clonally connected and therefore genetically the same (except for random somatic mutations). My tip and internode cuttings came from across the bunch.

Four analyses were carried out to untangle the genetic versus plastic components of variation among the 35 populations, and the interaction between the genotypes and environment (GxE). The null hypothesis for each test was that there would be no statistically significant difference among the populations for performance measures within each garden separately and for the GxE interactions.

First, I created histograms, normal Q-Q plots, and residuals vs fitted plots to visually assess patterns of trait distribution among the clones of all populations. I found that the statistical distributions were all normally distributed except for five traits: above ground dry weight, average largest leaf length and width, longest horizontal shoot and internode length. These five trait values required a log transformation to satisfy the assumptions of homoscedasticity and normality for the analyses.

To test for genetic differences among populations, I used an analysis of variance (ANOVA) on each of the 13 performance traits for each population, in each of the two gardens (Ilam and Cass) separately. As well as indicating whether differences were genetic or plastic, this indicated whether the environment experienced by plants at the separate common gardens affected phenotypic expression.

In order to explore the GxE interactions among key traits, I regressed the values of each trait for each population in Ilam against the corresponding values at Cass. From this I created a coefficient of determination (R^2) table. If a trait has a large R^2 value, the 'better the fit' or stronger the relationship between the performance measures at Ilam and at Cass. R^2 is an estimate which measures the ability of the model to predict an observation (Tjur, 2009).

A limitation to ANOVAs is that they don't allow for random vs fixed effects (parameters that do not vary). In order to be more accurate in my analyses, I also ran a series of linear mixed-effects models (LMM) which included random and fixed effects. Garden location was treated as a fixed effect in the model while region and population were treated as random effects. Unlike ANOVA, LMMS account for the multiple observations within a single plant and plants observed in multiple gardens (Boisgontier & Cheval, 2016). I used the function lme in the R package nlme (Bates *et al.*, 2015). Because the dataset was unbalanced (in this instance, an unequal number of individuals per population), I used a restricted maximum likelihood (REML), because it is less biased than the corresponding maximum likelihood (van Kleunen & Fischer, 2008; Bolker *et al.*, 2009). A Type I error was controlled for using the Bonferroni correction. The LMM table did not add much more to my understanding and I was able to better visualize my data using ANOVA. Consequently, I have not presented the LMM table in this chapter, however it can be found in the appendix (Appendix C – E).

Finally, I used a two-way analysis of covariance (ANCOVA) to determine the source of the observed variation. This model included region, population and the interation effect, region*population. The primary use of a two-way ANCOVA is to understand if there is an interaction between region and population on performance traits in *Erythranthe guttata*.

Here I present only statistically significant results in this chapter. Full analyses, including non-significant results, can be found in the appendix (Appendices A-F).

All statistical analyses were undertaken using R statistical software version 3.5.1 (R Core Team, 2013).

2.3 Results

The analysis of variance (ANOVA) indicated that there were significant ($p < 0.05$) differences in performance traits among the populations within both the Ilam and Cass gardens (Figure 2.8 and Table 2.6). Moreover, plants of the same genotype grown at Ilam tended to have less dry weight and shorter, narrower leaves compared to plants grown at Cass (Figures 2.8a and 2.8b). However, plants of the same genotype grown at Ilam tended to have longer horizontal shoots (Figures 2.8c and 2.8d) and a higher concentration of anthocyanin than plants grown at Cass.

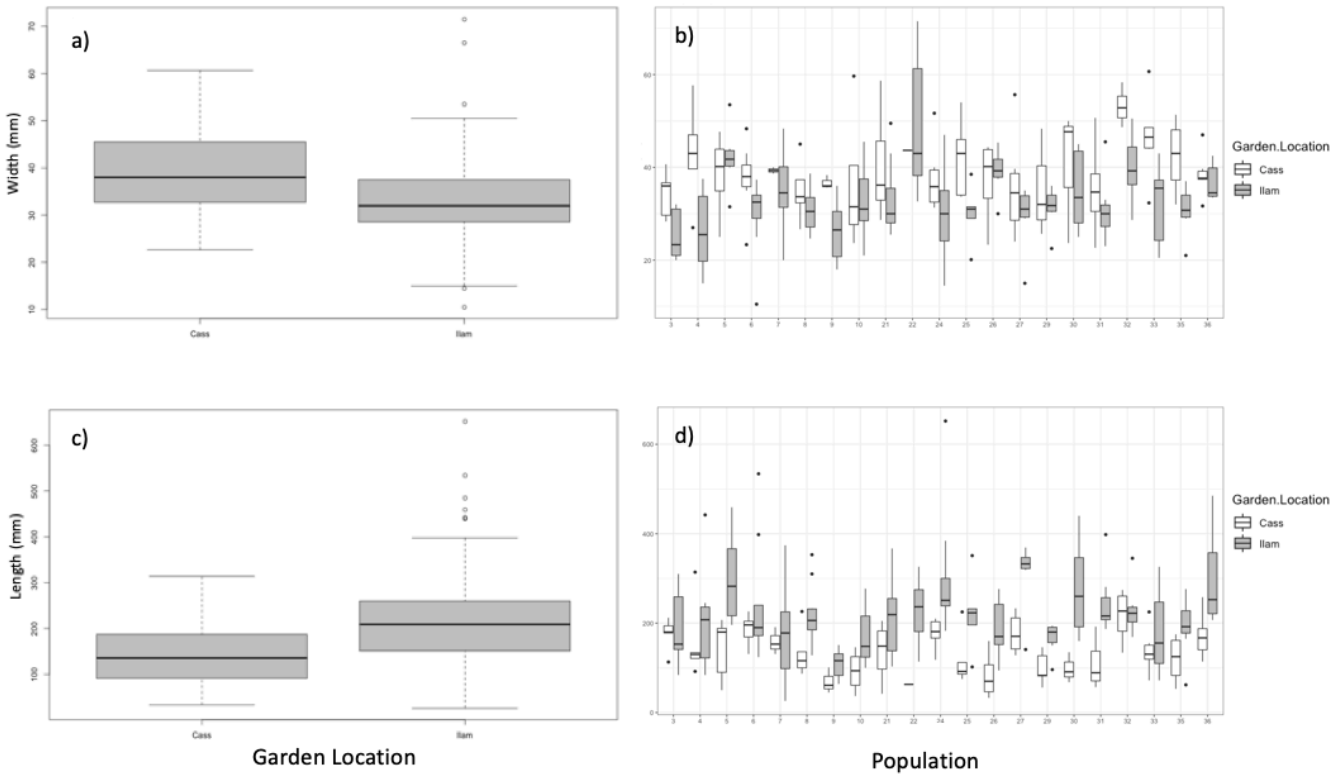

Figure 2. 8: Boxplots of performance measures by common garden site and by population. a) boxplot of the average largest leaf width for Cass and Ilam separately; b) boxplot of the average largest leaf width for all of the populations that were grown at both Cass (white) and Ilam (grey) separately; c) boxplot of the longest horizontal shoot for Cass and Ilam separately; d) boxplot of the longest horizontal shoot for all of the populations that were grown at both Cass (white) and Ilam (grey) separately.

Table 2. 6: Analysis of variance results showing only the significant differences in performance measures among populations at both Ilam and Cass common gardens.

**Significant ($p < 0.05$)*

Performance Measure	Ilam			Cass		
	F value	df	p value	F value	df	p value
Log Above Ground Dry Weight	2.4	137	**0***	1.54	79	0.09
Log Average Largest Leaf Length	2.83	137	**0***	0.83	79	0.67
Log Average Largest Leaf Width	2.75	137	**0***	1.02	79	0.45
Log Longest Horizontal Shoot	2.79	136	**0***	2.6	78	**0***
Longest Vertical Shoot	2.29	77	**0.01***	0.96	33	0.52
Log Internode Length	3.02	137	**0***	1.45	79	0.13
Anthocyanin Score	3.57	137	**0***	2.86	79	**0***
Jdate First Bud	1.25	19	0.26	2.54	30	**0.01***
Max Bud Number	1.52	140	0.08	5.3	79	**0***

Overall, there was a strong correlation between mean trait values at Ilam and Cass, so that their trait value at Cass could be predicted from their trait value at Ilam (Table 2.7). By far, the majority of traits across all populations indicated a strong relationship ($R^2 > 0.7$) (Moore *et al.*, 2013). However, I also observed a genotype by environment (GxE) interaction in some of the clones in my results. That is, in some cases different clones responded differently to the same change in the environment. I observed a GxE interaction across seven population:trait combinations (Table 2.7).

Table 2. 7: Coefficient of determination (R^2) table for the proportion of variance between the two common gardens for ten performance measures. Weak relationships ($R^2 < 0.4$) are highlighted in bold.

Population	Above Ground Dry Weight	Average Largest Leaf Length	Average Largest Leaf Width	Longest Horizontal Shoot	Longest Vertical Shoot	Internode Length	Anthocyanin Score	Jdate First Bud	Jdate Max Bud	Max Bud Number
3	0.667	0.655	0.804	0.751	**0.399**	0.771	0.89	NA	NA	NA
4	0.826	0.832	0.892	0.872	0.668	0.945	0.961	0.518	0.554	0.444
5	0.953	0.663	0.861	0.665	0.998	0.976	0.824	NA	NA	NA
6	0.885	0.748	0.862	0.637	0.609	0.736	0.493	0.471	0.44	0.722
7	0.995	0.955	0.75	0.848	NA	0.957	NA	NA	NA	NA
8	0.682	0.989	0.887	0.86	0.957	0.753	0.519	NA	NA	NA
9	0.996	**0.374**	0.509	0.609	NA	0.71	0.942	NA	NA	NA
10	**0.244**	0.768	0.579	0.915	NA	0.788	0.727	NA	NA	NA
21	0.646	0.9	0.736	0.907	NA	0.809	0.741	NA	NA	NA
24	0.877	0.746	0.613	0.931	NA	0.902	0.806	0.987	0.945	0.481
25	0.574	0.942	0.775	0.812	0.917	0.529	0.828	0.519	0.818	0.893
26	0.958	0.932	0.97	0.62	NA	0.524	0.474	NA	NA	0.75
27	0.688	0.601	0.533	0.482	NA	0.813	0.91	NA	NA	NA
29	0.73	0.844	0.666	0.725	0.802	0.836	0.667	0.75	0.59	0.992
30	0.691	0.837	0.65	0.906	NA	0.996	NA	NA	NA	NA
31	0.761	0.813	0.785	0.9	0.652	0.878	**0.276**	NA	NA	NA
32	0.999	0.908	0.894	0.933	NA	0.619	NA	NA	NA	NA
33	0.961	0.781	0.627	0.95	0.446	0.88	0.529	**0.018**	**0.335**	0.712
35	0.687	0.704	0.692	0.749	NA	0.859	0.889	NA	NA	NA
36	0.972	0.887	0.664	0.877	0.791	0.766	NA	0.528	**0.25**	NA

The two-way analysis of covariance (ANCOVA) highlighted significant genetic differences in the performance measures between regions and populations ($p < 0.05$) (Table 2.8). I found a significant difference between garden location across seven performance traits: dry weight, leaf length, leaf width, horizontal shoot, anthocyanin score, jdate max bud and flower depth. This was evidence of a GxE interaction. I found a significant difference among populations for all performance measures except: jdate max bud, flower height and flower width. This result was further evidence of the effect of different genotypes at each population.

A significant garden location and population effect was only found for jdate first bud. The effect of this interaction explained 18% of the variance, while the difference between the two garden locations and difference between the populations separately explained 2% and 29%, respectively.

Table 2. 8: Two-way analysis of covariance of garden location and population only showing significant differences in performance measures among populations. Degrees of freedom for the residual is: 297 for log above ground dry weight, log average largest leaf length and width, internode length and anthocyanin score; 292 for log longest horizontal shoot; 133 for vertical shoot; 87 for jdate first bud; 86 for jdate max bud; 310 for max bud number; 61 for largest flower height, depth and width.

**Significant (p < 0.05)*

Performance Measure		**Source of Variation**		
		Garden Location	Population	GL*Population
Log Above Ground Dry Weight	F-statistic	22.48	5.35	1.20
	p-value	**<0***	**<0***	0.26
	% Variance	4	35	5
Log Average Largest Leaf Length	F-statistic	30.86	4.48	0.79
	p-value	**<0***	**<0***	0.72
	% Variance	6	31	3
Log Average Largest Leaf Width	F-statistic	63.41	6.08	0.85
	p-value	**<0***	**<0***	0.65
	% Variance	11	35	3
Log Longest Horizontal Shoot	F-statistic	30.23	6.06	1.17
	p-value	**<0***	**<0***	0.28
	% Variance	5	37	4
Longest Vertical Shoot	F-statistic	1.84	1.91	1.21
	p-value	0.18	**≤0***	0.26
	% Variance	1	27	10
Log Internode Length	F-statistic	1.99	4.93	1.35
	p-value	0.16	**<0***	0.15
	% Variance	0	34	5
Anthocyanin Score	F-statistic	7.79	12.21	1.53
	p-value	**0.01***	**<0***	0.07
	% Variance	1	55	4
Jdate First Bud	F-statistic	3.56	1.90	2.14
	p-value	0.06	**0.01***	**0.02***
	% Variance	2	29	18
Jdate Max Bud	F-statistic	12.12	1.24	1.21
	p-value	**≤0***	0.23	0.28
	% Variance	8	22	12
Max Bud Number	F-statistic	1.05	2.20	1.57
	p-value	0.31	**≤0***	0.06
	% Variance	0	18	8
Largest Flower Depth	F-statistic	4.10	1.17	1.09
	p-value	**0.05***	**0.30***	0.38
	% Variance	4	28	13

2.4 Discussion

Previous studies on *Erythranthe guttata* in its native range of North America have shown that populations occupying different habitats elicit pronounced phenotypic differences (Chalker-Scott, 1999; Hall & Willis, 2006; Murren *et al.*, 2009; Lowry *et al.*, 2012; Peterson *et al.*, 2016). Overall, it has been demonstrated that in different habitats, morphological, physiological, phenological and life-history traits are affected. This includes habitats which vary in photoperiod, temperature, water availability and altitude. Through allozyme analysis, van Kleunen and Fischer (2008) were able to show that most of the genetic variation in the native range of *E. guttata* has already been introduced to Scotland and New Zealand. This indicates that there is sufficient genetic variability in New Zealand populations to adapt to local selective pressures.

The purpose of the common garden experiment was to determine if phenotypic differences observed among populations of *E. guttata* across New Zealand were a result of genetic differentiation or phenotypic plasticity. If this variation in phenotype was the result of genetic differentiation, the populations in the two common gardens should maintain phenotypic differences reflective of differences in genotype. Genetic differences among populations could reflect local adaptation, although I have not tested for that. Alternatively, it may reflect multiple introductions. Natural selection and local adaptation favours survival of individuals with genotypes best adapted to local environmental conditions fostering the continuation of those genetic lines (Darwin, 1859). Over generations, this would lead to genetically-distinct populations among New Zealand habitats. A lack of significant variation in performance measures among populations in both common gardens suggests a role of phenotypic plasticity.

The 35 populations of *Erythranthe guttata* from across New Zealand, used in this study showed patterns of both genetic differentiation and phenotypic plasticity. Statistical differences between one or more of the populations in each of the two common garden sites separately was evidenced across nine performance measures (Table 2.5), implying genetic variability in these traits. The non-significant result I found for four performance measures (jdate max bud and the three flower measurements (Figure 2.6) was surprising; this is because

clearly some flowers were visibly smaller than most and these small flowers were associated with population 6. It is likely that the non-significant result is because I had so many of the 'common' large flower type and relatively few of the small flower type; in this year of the study, relatively few plants flowered. In the second year of the experiment, many more plants flowered and there was a significant difference. An interaction between the population of origin and the common garden site was found for only one performance measure, jdate first bud. This indicates a strong genotype by environment (GxE) interaction for this performance measure; there are differences among populations which maintain different responses in the two common garden sites and is indicative of an ability to adapt (Figure 2.9).

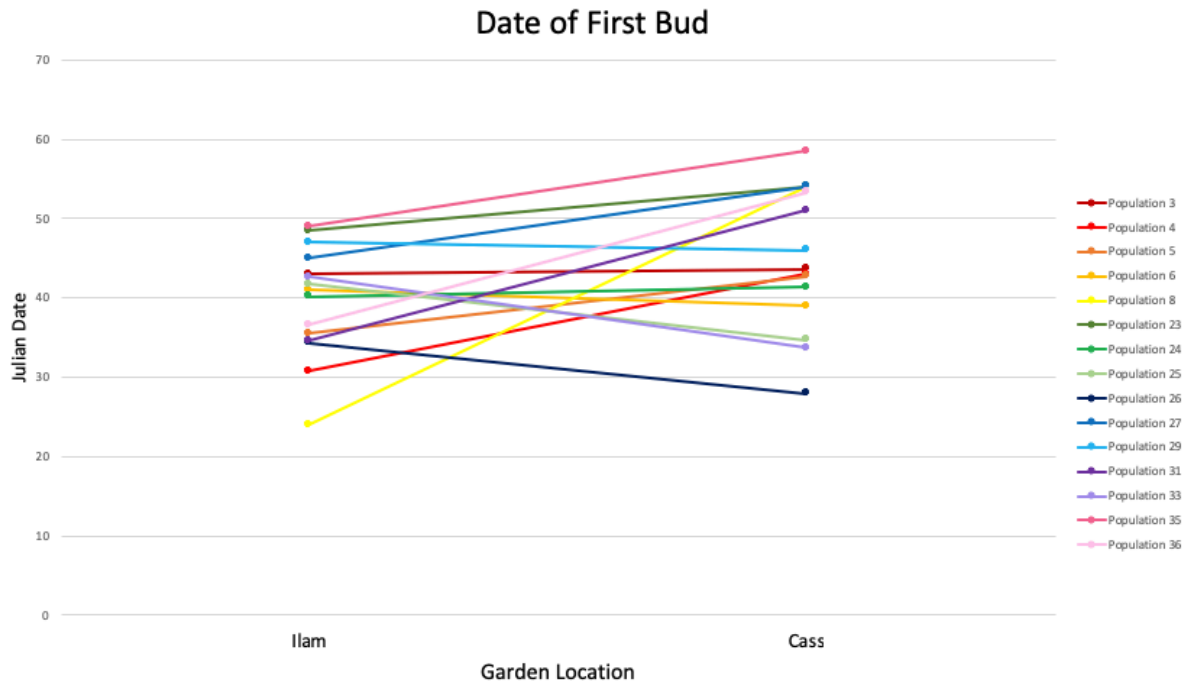

Figure 2. 9: Distinct genotypes of Erythranthe guttata are represented by the coloured lines. Genotypes exhibit variable responses in the separate gardens for jdate first bud suggesting an adaptation of the jdate first bud to environmental change.

At each population, I observed strong correlations between Ilam and Cass across genotypes for the majority of performance traits. This illustrates the environmental component of the variation, with a range of traits showing correlated responses to the same environmental change. However, in six of the populations across six traits (seven population:trait combinations) a GxE interaction could be observed. This suggests that almost all genotypes

(clones/populations) respond in the same way to the same change in environment – there is no GxE interaction.

Genotypic variability and plasticity appear to be the driving forces behind phenotypic expression. As Figure 2.9 illustrates for the jdate first bud, a single genotype changes its phenotype according to environment. Overall, the environment at Cass led to an increase in the above ground dry weight and narrower leaves. The key point is that all genotypes tended to respond to the Cass environment in the same way, which suggests no GxE interaction (except for jdate first bud) (Table 2.6).

Overall, the results emphasise genetic variability in New Zealand populations of *E. guttata*. This genetic variability is the primary driver for the observed differences in phenotype among populations. However, phenotypic plasticity can be observed to influence the observed phenotype in unison with genetic differences, but to a lesser degree. There was very little evidence of a GxE interaction in the *E. guttata* populations.

The co-occurrence of genetic variation and phenotypic plasticity is not uncommon in the literature. The interplay between phenotypic plasticity and genetic variability has been reported frequently with evidence suggesting a mutual benefit (Pigliucci, 2007; Liao *et al.*, 2016). Phenotypic plasticity may even facilitate genetic differentiation in response to environmental variability (Sexton *et al*, 2002; Bennington *et al*., 2012). The mutual benefit would make sense in the current study with previous research indicating high genetic diversity in New Zealand *E. guttata* populations as well as the salient role of phenotypic plasticity in range expansion.

The mix of statistically significant and non-significant differences between performance measures at Ilam and Cass further the emphasises the effect of genetic differentiation and phenotypic plasticity. The different gardens impose different selective pressures on the plants. Therefore, a fixed phenotype, such as observed for the dry weight, leaf length, leaf width, horizontal shoot, vertical shoot, internode length, anthocyanin score, jdate first bud and max bud number was suggestive of genetic controls. A fluid phenotype, as observed for the jdate max bud and all three flower measurements, was typical of plasticity.

The linear mixed-effects model (LMM) not presented in this chapter showed a discrepancy to the ANOVA with less significance amongst traits i.e. showed that only leaf length, leaf width and horizontal shoot were significant. This suggested that fewer traits were genetically controlled.

While beginning to untangle phenotypic differences due to genotype and phenotypic plasticity, this study did not include the influence of maternal effects. The inclusion of maternal effects was outside the breadth of this research; however, they have the ability to confuse the interpretation of single year common garden studies. They can influence phenotypic expression without altering genotype, such as epigenetic inheritance (Latzel & Klimesova, 2010). Collecting data over multiple years (chapter 4) will begin to disentangle genotypic differentiation and phenotypic plasticity from maternal effects.

2.5 Summary

Populations of *Erythranthe guttata* grown in two separate common gardens displayed marked phenotypic differences. The common garden experiment tested 35 populations of *E. guttata* from across seven geographically-distinct regions in New Zealand. The objective of this experiment was to determine whether phenotypic variation seen in the field is the result of phenotypic plasticity or genetic differentiation. Plant performance showed significant statistical differences among gardens and among populations, suggestive of genetic variability. However a statistically significant difference could not be found across all performance measures. This suggests that coinciding with genetic differentiation, phenotypic plasticity is also regulating the variation in phenotypic expression. A genotype by environment interaction could only be found for jdate first bud.

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Chapter 3

Evidence for latitudinal trends across New Zealand populations of *Erythranthe guttata*?

3.1 Introduction

Erythranthe guttata is an exotic invasive species threatening the indigenous biodiversity of New Zealand riparian systems. Its successful invasions can be attributed to its tolerance to a wide variety of habitats from running water to periodically dry ditches. In New Zealand it occurs from Whangarei in the North Island to Bluff in the South Island. Common garden experiments (Chapter 2) have found that *E. guttata* individuals show some degree of genetic variation among populations as well as utilizing phenotypic plasticity to produce a multitude of phenotypes in heterogenous environments.

Latitudinal trends are be observed across the globe. A latitudinal trend is an observable pattern from the poles to the Earth's equator. A well-studied trend is the increase in biodiversity from the poles (high latitudes) to the tropics (Ohlemuller & Wilson, 2000; Kinlock *et al.*, 2018). Environmental variables such as temperature, solar radiation, water availability, altitude and photoperiod vary along latitudinal gradients and these all impact plant growth and reproduction (McMillan, 1967; Li *et al*., 1998; Olsson & Agren, 2002; Willis & Hulme, 2002; Griffith & Watson, 2005; van Kleunen & Fischer, 2008; Bull-Herenu & Arroyo, 2009; Michalski *et al.*, 2017; Qiu *et al*., 2018).

Numerous studies have shown latitudinal trends in relation to plant phenology, life history, morphology and physiology (Winn & Gross, 1993; Griffith & Watson, 2005). Plants at lower latitudes tend to have a high reproductive output than plants at higher latitudes (De Frenne *et al*., 2011). Differences in temperature (colder at higher latitudes and warmer at lower latitudes) can contribute to observed variation in flowering characteristics, for example delayed flowering at higher latitudes (Phillips *et al*., 1983; Kollmann & Banuelos, 2004; De Frenne *et al*., 2011; Lowry *et al*., 2014: Qiu *et al*., 2018). With increasing latitude, the growing

season shortens (Hall *et al*., 2007) thereby creating a strong selection pressure for plants with delayed flowering (Ofir & Kigel, 2006), shorter flowering season and increasing flowering synchrony, thereby decreasing the risk of low-temperature damage (Griffith & Watson, 2005; Lowry *et al*., 2014; Qiu *et al*., 2018). For species producing a capitulum, such as *Chaetanthera moenchioides*, later flowering in populations from higher latitudes is correlated with the production of more flowers per capitulum (Bull-Herenu & Arroyo, 2009).

Seed and fruit size is another reproductive feature affected by latitude. Increasing latitude has been correlated with a decrease in seed and fruit size in a diverse range of species including *Arabidopsis thaliana* and *Glycine* species, where seed mass decreased with increasing latitude (Li *et al.*, 1998; Westoby *et al.*, 2002; Moles & Westoby, 2003; Murray *et al*., 2003; Moles *et al.*, 2007). A synonymous result was found in species of the genus *Quercus* which produced smaller acorns at higher latitudes than conspecifics at low latitudes (Aizen & Woodcock, 1992). Contradictory to the plethora of research, seed and fruit size has been shown to increase with increasing latitude in the small shrub, *Crataegus monogyna* (Sobral *et al*., 2013).

Associated with delayed flowering, plants at higher latitudes tend to allocated more energy to vegetative growth before a flowering event, thereby enabling plants to be larger prior to beginning reproduction (Kollmann & Banuelos, 2004; Ofir & Kigel, 2006; van Kleunen & Fischer, 2008). At higher latitudes, plants have also been shown to decrease their relative growth rate (Li *et al*., 1998; Moles *et al.*, 2009) but increase their daily growth rate during the summer months, possibly to compensate for the shorter growing season (Griffith & Watson, 2005). Individuals from low latitudes have also been shown to dedicate a greater proportion of biomass into root growth than conspecifics from higher latitudes (Reinartz, 1984).

Water availability along a latitudinal gradient is an important environmental variable in the elicitation of particular phenotypic and phenological traits. A latitudinal gradient for water availablity has been identified with increased precipitation and decreased evapotranspiration with increasing latitude (Truscott *et al.*, 2006; Bull-Herenu & Arroyo, 2009). Dessication of *Chaetanthera moenchiodes*, is more likely at low latitudes and is responsible for the shorter flowering time and dictating the time to senescence (Bull-Herenu & Arroyo, 2009). Arid

environments tend to support plant species which show early flowering so as to avoid wasting reproductive effort during the harsh conditions imposed by drought (McMillan, 1967; Wu *et al*., 2010).

3.1.1 Latitudinal trends in populations of *Erythranthe guttata*

Erythranthe guttata occurs across a range of latitudes (Hall & Willis, 2006) and in its native range of the United States, shows latitudinal trends. There *E. guttata* is geographically widespread, with wide tolerance and performance breadths (Sheth & Angert, 2014). In the US, *E. guttata* tends to reproduce more via vegetative reproduction and autonomous self-fertilization with increasing latitude. Conversely, with decreasing latitude, sexual reproduction is more common with the production of more flowers and a decrease in total stolon length (van Kleunen & Fischer, 2008). Interestingly, it was observed by van Kleunen and Fischer (2008) that plant height of *E. guttata* was not affected by latitude, which is in contrast to the findings of Kollmann and Banuelos (2004) for *Impatiens glandulifera* and Olsson *et al*. (2002) for *Lythrum salicaria*, both invasive floral species. Water availability across a latitudinal and ecogeographic gradient has also been shown to strongly influence phenotypic and phenological differences in *E. guttata* (Wu *et al*., 2010). *E. guttata* prefers habitats with a reliable moisture supply (Kiang & Hamrick, 1978), however individuals from drier or drought-prone environments show early flowering and rapid maturation, enabling seed production before drought conditions are imposed (Wu *et al*., 2010).

3.1.2 Latitudinal trends in New Zealand plant species

Research on latitudinal gradients in New Zealand provides ambivalent results. In agreement with research outside of New Zealand, Ohlemuller and Wilson (2000) identified a latitudinal trend in species richness among New Zealand temperate rainforests. They observed an 11% decrease in species richness per one degree of increasing latitude. However, at the level of individual species, results mixed. Flowering has been observed as occurring earlier in populations of the Australia and New Zealand native, *Leptospermum scoparium,* at increased latitudes, (Harris, 2002) likely a response to the shorter growing period at southern latitudes. In contrast, *Agrostis capillaris* has not evolved latitudinal ecotypes, despite its presence in New Zealand for over 130 years (Rapson & Wilson, 1992).

3.1.3 Objective two

My main objective in this chapter was to determine whether New Zealand populations of *Erythranthe guttata* show clinal variation associated with a latitudinal gradient. My approach was to use a common garden experiment to look for genetic differences in populations that vary by latitude. My null hypothesis was that there is no variation in New Zealand populations of *E. guttata* along a latitudinal gradient.

3.2 Methods

3.2.1 Experimental Background

Exotic plant species have been shown to elicit patterns of invasion on both global and regional scales (Willis & Hulme, 2002; Kollmann & Banuelos, 2004). A common method applied in studies of latitudinal trends in invasive species is the utilisation of a common garden (Olsson & Agren, 2002). This involves sampling populations from multiple different habitats which vary in environmental conditions and translocating them to a "common garden" where all environmental conditions can either be controlled or kept constant across all samples. In effect, each individual sample experiences identical growing conditions (e.g. light, water, nutrients, and temperature).

3.2.2 Population locations and sampling

I used the same populations of *Erythranthe guttata* and sampling methods as described in Chapter 2 (Table 2.2).

3.2.3 Common garden set up

I used the same common gardens described in Chapter 2 (Table 2.4).

3.2.4 Performance measurements

I used the same performance measures as were described in Chapter 2 (Table 2.5).

The main performance indicator for a latitudinal trend was phenology. Along a latitudinal gradient, environmental factors affecting phenology, such as temperature and photoperiod,

are known to vary causing obvious shifts in phenological features (Olsson & Agren, 2002; Kollmann & Banuelos, 2004; Wu *et al.*, 2010).

Other latitudinally affected performance measures include morphological features such as biomass, plant height, length and internode length (Li *et al.*, 1998; Olsson & Agren, 2002; Kollmann & Banuelos, 2004; Ofir & Kigel, 2006).

3.2.5 Analysis

To look for evidence of latitudinal patterns in the data, I first used a multivariate principal component analysis (PCA) using all of the performance indicators across all 35 populations. A PCA compresses multi-dimensional data, in this case, multiple performance measures, into a small dataset that can be visualized two-dimensionally. The axes are represented by principal components (PCs), values given to a set of variables which are linearly uncorrelated. Each individual on a biplot is represented by a single dot that represents the position of each 'clone' in relation to the first two PCs. The biplot also illustrates how the performance traits map onto the first two PCs. On a biplot they are vectors (arrows) originating from the centre and pointing in the direction in which high values of a trait move. I ran the PCA using all 13 performance measures (Table 2.5).

In addition, I used the output from the analysis of variance (ANOVA) (Chapter 2) to explore any effect of latitude in differences among populations in performance traits. I produced a bar chart of each of the populations average jdate first bud and internode length, and ranked them (either from earliest to latest to bud or shortest to longest internode length). I did this for each of the two common gardens separately.

Irrespective of a relationship between performance traits and latitude, it is important to determine which (if any) environmental variables may be selecting for phenotypic expression of performance traits. To answer this question, I created a correlations table between all 13 performance measures and 12 of the environmental variables presented in Table 2.3. A strong relationship between performance measure and any environmental variables would indicate that phenotypic expression of that performance trait may be determined by the environmental variable to which it shares a strong relationship.

Finally, I produced a three-way analysis of covariance (ANCOVA) to analyse the source of variation (garden location, latitude and region) in all 13 performance traits. In my analysis, the independent variables were garden location, latitude and region and the interaction effects were: garden location*latitude, garden location*region, latitude*region and garden location*latitude*region. In this chapter I present only six key performance traits known to potentially vary with latitude (Table 3.1).

Table 3. 1: Six key performance traits known to potentially show latitudinal trends in Erythranthe guttata *and similar species.*

Performance Trait	Reference
Above ground dry weight	Li *et al*. (1998)
Longest horizontal shoot	Kollmann & Banuelos (2004)
Longest vertical shoot	Kollmann & Banuelos (2004)
Internode length	Lowry *et al.* (2008)
Jdate first bud	Olsson & Agren (2002); Kollmann & Banuelos (2004); Ofir & Kigel (2006)
Max bud number	Olsson & Agren (2002)

Full analyses, including performance traits not presented in the results, can be found in the appendix (Appendix G).

All statistical analyses were undertaken using R statistical software version 3.5.1 (R Core Team, 2013).

3.3 Results

I found almost no indication of latitudinal trends across 13 performance measures from any of the analyses.

At Ilam, PC1 accounted for 37.6% of the total variance and PC2 accounted for 14% (Table 3.2). Cumulatively, the first two PCs accounted for 51.6% of the total variation. At Cass, PC1 accounted for 35.7% of the total variance and PC2 accounted for 13.9% (Table 3.2). Cumulatively, the first two PCs accounted for 49.6% of the total variation.

The principal component analysis (PCA) biplot of the first two PCs, at each garden, illustrated no obvious relationship between performance measures and latitude. Extensive scattering of individuals across the biplot at both Ilam and Cass gardens (Figure 3.1), is indicative of high levels of phenotypic variation without any latitudinal sorting.

Table 3. 2: Principal component analysis table for the explained variation among regions at Ilam and Cass separately, across 13 performance measures. "PC" represents principal component.

Garden Location		PC1	PC2	PC3	PC4
Ilam	StdDev	1.787	1.090	0.984	0.863
	Variance	0.376	0.140	0.114	0.088
	Cumulative Var.	0.376	0.516	0.630	0.718
Cass	StdDev	1.836	1.144	1.053	0.941
	Variance	0.357	0.139	0.117	0.094
	Cumulative Var.	0.357	0.496	0.613	0.707

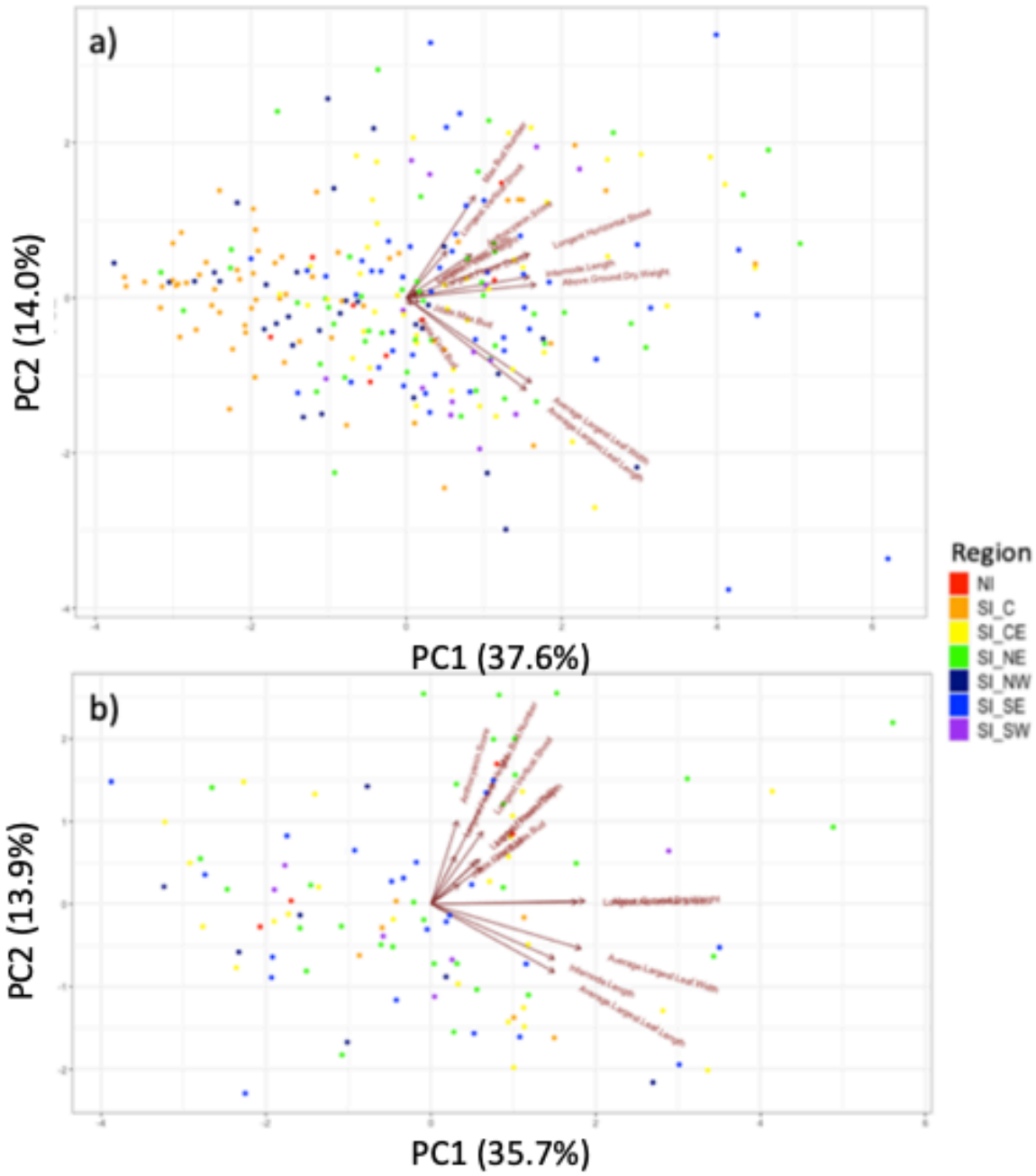

Figure 3. 1: Principal component analysis (PCA) biplots delineating principal component 1 and principal component 2. Region is represented by the colour-coded identifiers; North Island (NI), South Island Central (SI_C), South Island Central East (SI_CE), South Island North East (SI_NE), South Island North West (SI_NW), South Island South East (SI_SE), South Island South West (SI_SW). The x-axis represents PC1 and the y-axis represents PC2. a) PCA for plants grown at Ilam; b) PCA for plants grown at Cass.

The bar charts of jdate first bud and internode length, created from the ANOVA output are presented in Figures 3.2 and 3.4. Ranking the average jdate first bud for Ilam and Cass separately failed to show clustering of populations by region (Figures 3.2 and 3.4).

At Ilam, there was a difference of 25 days between the first and last populations to produce first buds (Figure 3.2 and 3.4). Within this variation, my results showed no latitudinal trend to which of the populations produced buds (on average) early or late. For example, populations from three regions, SI_C, SI_CE and SI_NE, produced buds early, with a mean jdate to first bud of 24 days (Figure 3.2). SI_C was also home to the second to last population to produce buds (μ = 45 days). Population 35, from the far North Island was the last population to develop buds (μ = 49 days).

At Cass, there was a difference of 30 days between the first and last populations to produce the first bud (Figure 3.2 and 3.3). Population 35 was again the last to develop buds but did so on average ten days later than at Ilam (μ = 58.5 days). Of note was that population 8 was first equal to develop buds in Ilam (μ = 24 days) but at Cass it was the second to last (μ = 54 days). Population 26, from the SI_SE is the first to develop buds at the Cass garden (μ = 28 days).

Populations of different regions are scattered across the bar charts for internode length at Ilam and Cass (Figure 3.4).

At Ilam, there was a difference of 48 mm between the shortest and longest population internode lengths (Figures 3.4 and 3.5). Within this variation, my results showed no latitudinal trend in internode lengths. For example, population 17 from SI_C had the shortest internode lengths on average (μ = 8.75 mm), while population 36, also from SI_C had one of the longest average internode lengths (μ = 53.57 mm). Population 5, from SI_NE had the longest internode length on average (μ = 57.08 mm).

At Cass, there was a difference of 35 mm between the shortest and longest population internode lengths (Figures 3.4 and 3.5). Once again, population 36 had one of the longest average internode lengths (μ = 50.17 mm), while population 5 had a much shorter internode length (μ = 29.11 mm). The shortest internode length on average was found in population 9 from SI_NW (μ = 16.24 mm), and the longest internode length on average was found in population 32 from SI_CE (μ = 50.88 mm).

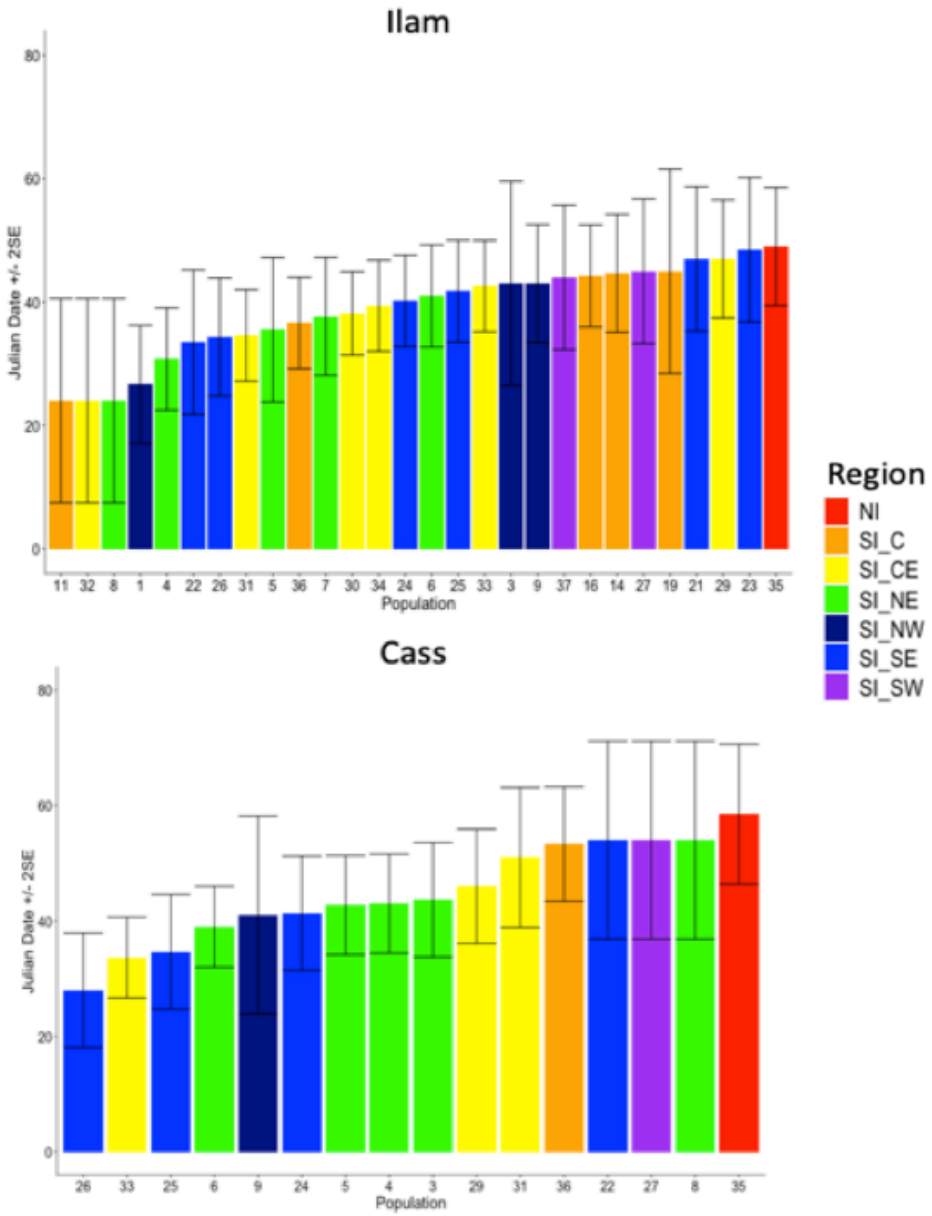

Figure 3. 2: The average jdate of the first bud for each population that developed buds at Ilam (above) and Cass (below) between January 1, 2018 and April 30, 2018. The regions are colour coded; North Island (NI), South Island Central (SI_C), South Island Central East (SI_CE), South Island North East (SI_NE), South Island North West (SI_NW), South Island South East (SI_SE), South Island South West (SI_SW). The y-axis represents the Julian Date +/- 2 standard error (SE) and along the x-axis are the populations.

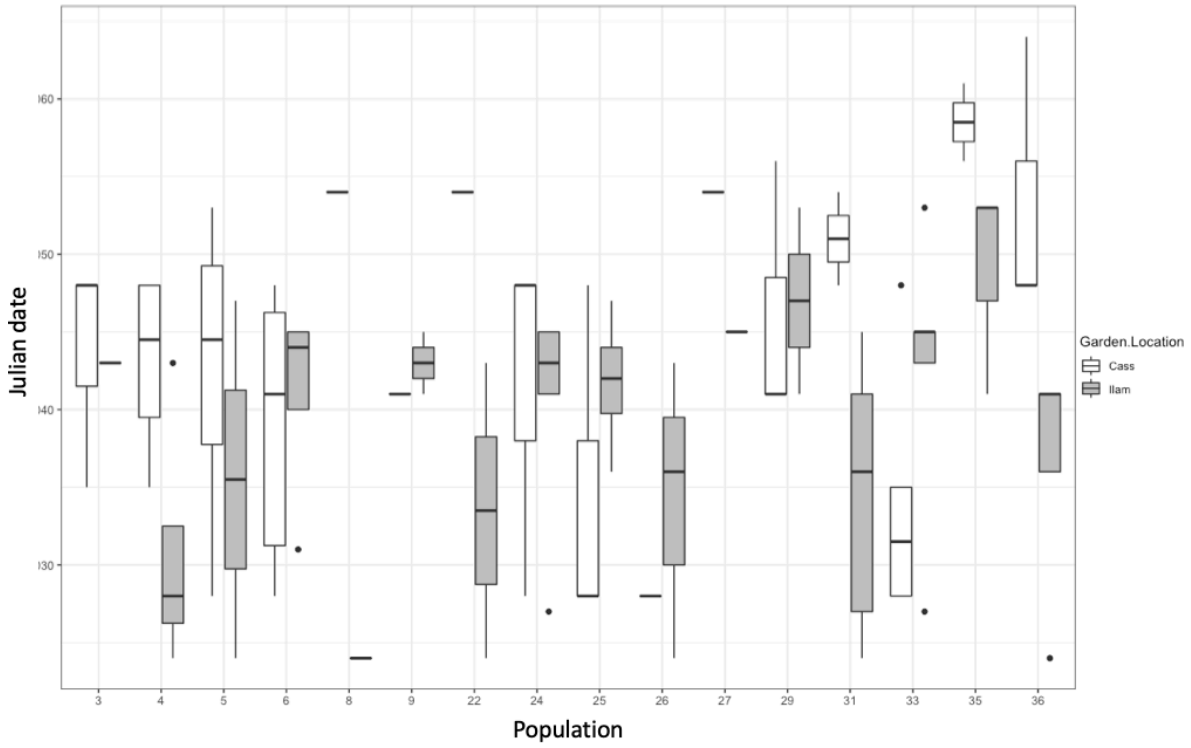

Figure 3. 3: Boxplot of the jdate first bud for only the populations that produced bud at both Ilam (grey) and Cass (white) separately.

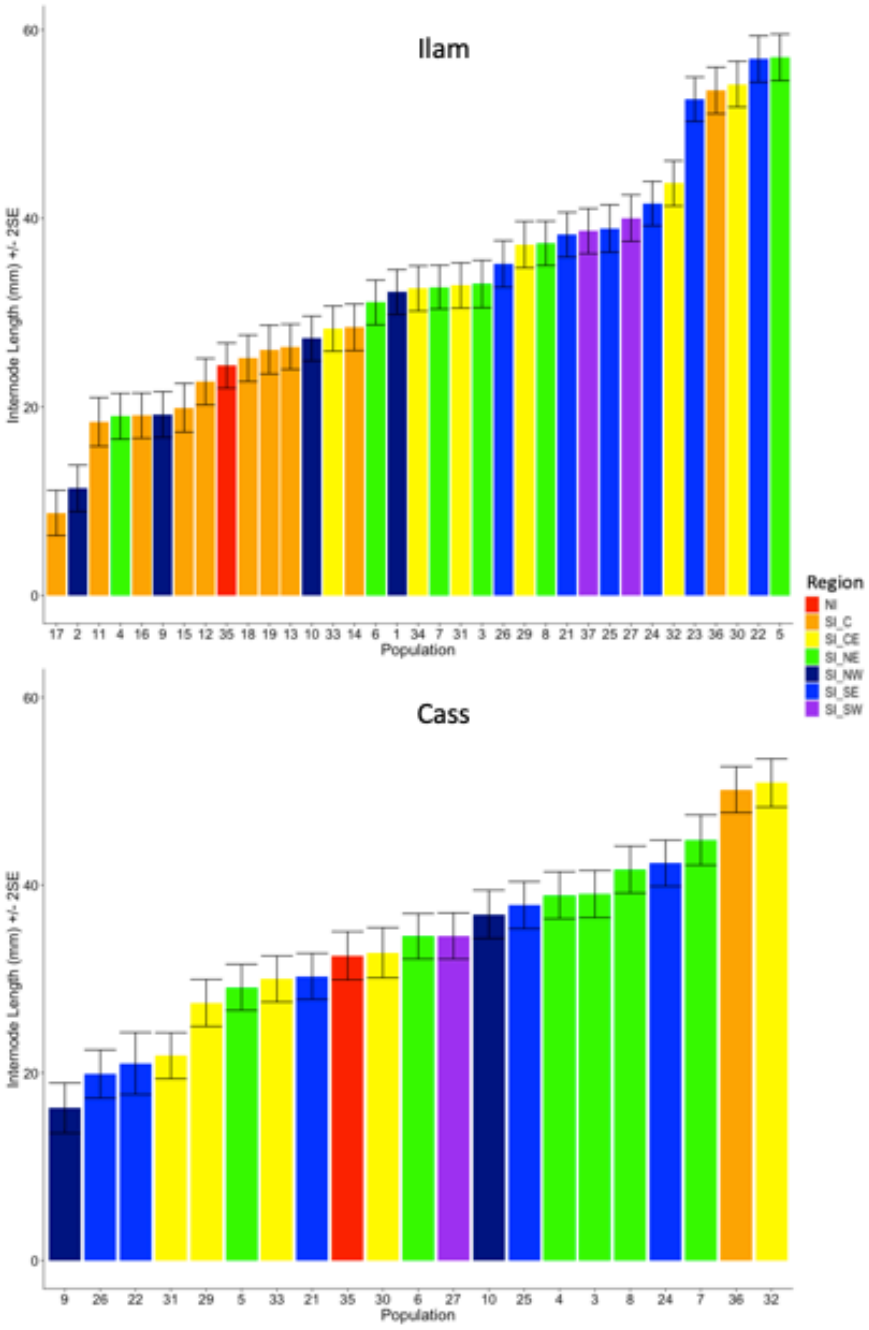

Figure 3. 4: The average internode length for each population that developed buds at Ilam (above) and Cass (below) between January 1, 2018 and April 30, 2018. The regions are colour coded; North Island (NI), South Island Central (SI_C), South Island Central East (SI_CE), South Island North East (SI_NE), South Island North West (SI_NW), South Island South East (SI_SE), South Island South West (SI_SW). The y-axis represents the Internode Length (mm) +/- 2 standard error (SE) and along the x-axis are the populations.

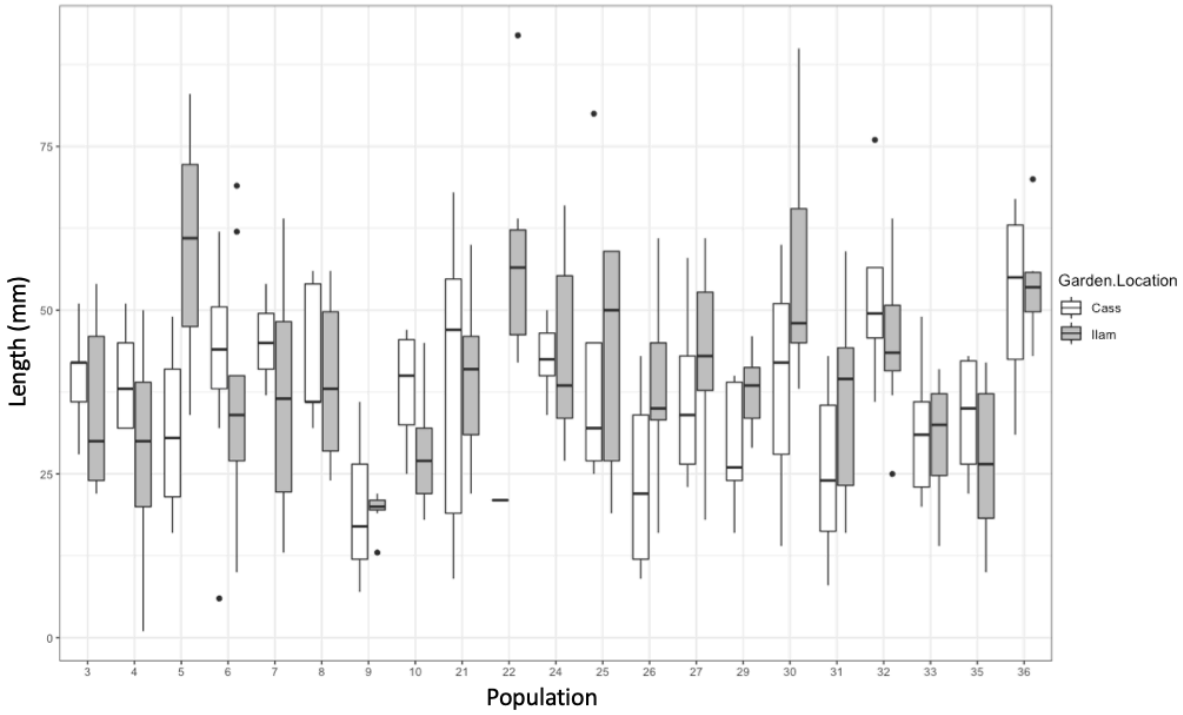

Figure 3. 5: Boxplot of the internode length for all of the populations that were grown at both Ilam (grey) and Cass (white) separately.

The correlations table of performance traits versus environmental variables are presented in Table 4.3. Based on other work (Moore *et al*. 2013), I classified a weak relationship as being R < 0.5. All of the correlations showed weak relationships between performance measure and environmental variables.

The three-way analysis of covariance (ANCOVA) (Table 3.4) was the only analysis to suggest any latitudinal trend, and this was not strong. On its own, I found a significant ($p < 0.05$) interaction between latitude and internode length ($p = 0.04$). However, from the ANOVA bar charts (Figure 3.4) it was difficult to observe any latitudinal trend based on regions alone. I can't say with any confidence what the trend in internode length is. The ANCOVA showed a significant two-way interaction between garden location and latitude for internode length and jdate first bud, garden location and latitude for max bud number, and latitude and region for dry weight and horizontal shoot. I found a three-way interaction effect between garden location, latitude and region for dry weight and jdate first bud. The significant three-way interaction represented 2% of the variance for dry weight and 10% of the variance for jdate first bud (Table 3.4).

Table 3. 3: Correlation table comparing the correlations of environmental variables with each of the thirteen performance measures.

Environmental Variable	Performance Measure												
	Above Ground Dry Weight	Largest Flower Height	Largest Flower Depth	Largest Flower Width	Average Largest Leaf Width	Average Largest Leaf Length	Longest Vertical Shoot	Longest Horizontal Shoot	Internode Length	Anthocyanin Score	Max Bud Number	Jdate Max Bud	Jdate First Bud
Latitude	-0.032	0.061	0.104	0.099	-0.025	0.018	-0.032	-0.095	-0.126	0.132	0.036	0.115	0.138
Annual Average Temperature	0.092	0.074	0.163	0.200	0.126	0.142	-0.009	0.004	-0.017	0.236	0.097	0.093	0.018
Average Temperature**	0.035	0.099	0.156	0.195	0.054	0.091	-0.003	-0.079	-0.113	0.126	0.084	0.101	0.018
Average Min Temperature**	0.159	0.058	0.152	0.215	0.186	0.172	0.018	0.092	0.065	0.285	0.120	0.080	0.029
Absolute Min Temperature**	0.180	-0.008	0.119	0.137	0.223	0.186	0.023	0.197	0.162	0.347	0.102	0.029	0.012
Average Max Temperature**	-0.107	0.103	0.127	0.138	-0.084	-0.013	-0.030	-0.215	-0.242	-0.078	0.022	0.119	0.039
Absolute Max Temperature**	-0.088	0.021	0.045	0.122	-0.128	-0.066	0.025	-0.158	-0.161	-0.220	0.022	0.166	0.081
Annual Average Rainfall	-0.029	-0.057	-0.049	-0.136	0.054	0.041	-0.093	0.110	0.185	0.117	-0.110	-0.091	0.033
Humidity**	0.064	-0.061	0.051	0.044	0.017	0.028	-0.006	0.076	0.148	0.058	0.028	-0.055	-0.027
Precipitation**	-0.030	-0.061	-0.059	-0.142	0.057	0.040	-0.090	0.112	0.188	0.111	-0.110	-0.095	0.029
Temperature at Collection	-0.104	0.065	0.043	0.064	-0.159	-0.174	0.190	-0.213	-0.307	-0.135	0.151	0.021	-0.091
Sunlight Hours	0.041	-0.020	0.026	0.055	-0.038	-0.045	0.056	0.082	0.054	-0.208	-0.014	0.083	-0.015

***Environmental averages for the growing period (September-March).*

Table 3. 4: Three-way analysis of covariance of garden location, latitude and region on six performance traits known to vary along latitudinal gradients. Degrees of freedom for the residual is 329, 324, 158, 329, 108 and 342 for log above ground dry weight, log longest horizontal shoot, longest vertical shoot, log internode length, jdate first bud and max bud number, respectively.

**Significant (p < 0.05)*

Source of Variation	Log Above Ground Dry Weight			Log Longest Horizontal Shoot			Longest Vertical Shoot			Log Internode Length			Jdate First Bud			Max Bud Number		
	F-statistic	p-value	% Var.	F-statistic	p-value	% Var.	F-statistic	p-value	% Var.	F-statistic	p-value	% Var.	F-statistic	p-value	% Var.	F-statistic	p-value	% Var.
Garden Location	19.99	**≤0***	4	25.65	**<0***	5	1.69	0.19	1	1.74	0.19	0	3.50	0.06	2	0.97	0.33	0
Latitude	1.21	0.27	0	1.11	0.26	0	0.10	0.75	0	4.35	**0.04***	1	2.60	0.11	2	0.24	0.63	0
Region	14.48	**<0***	19	15.04	**<0***	19	1.68	0.13	5	11.82	<0*	16	2.74	**0.02***	10	2.30	**0.03***	4
GL*Latitude	≤0	0.94	0	2.53	0.11	1	0.50	0.48	0	6.20	**0.01***	1	5.61	**0.02***	3	1.59	0.21	0
GL*Region	0.86	0.52	1	1.33	0.24	2	2.07	0.06	6	1.04	0.40	1	1.20	0.31	4	2.65	**0.02***	4
Latitude*Region	2.65	**0.02***	3	2.31	**0.04***	2	1.91	0.10	5	1.55	0.17	2	2.20	0.06	6	1.81	0.11	2
GL*Latitude*Region	3.85	**0.01***	2	1.68	0.17	1	1.09	0.36	2	1.25	0.29	1	8.25	**≤0***	10	0.52	0.67	0

3.4 Discussion

Overall my results from 35 populations from across New Zealand provide very little evidence of latitudinal trends in performance traits. For example, the PCA biplot (Figure 3.1) showed no clustering of populations by region (which was a loose proxy for latitude). In addition, neither did the bar charts from the ANOVA (Chapter 2; Figures 3.2 and 3.3) show any observable trends in either of the performance traits across the regions. However, the results of the three-way ANCOVA were less clear – they suggested that internode length does show a weak but significant effect of latitude (Table 3.4). To see what the trend in internode length was, I used the regions as a proxy for latitude, but this saw no detectable cline.

Using allozyme analysis and looking at morphology, van Kleunen and Fischer (2008) suggested that there was evidence of post-introduction adaptive evolution in the invasive ranges of *Erythranthe guttata*. Plants from the invasive ranges of Scotland and New Zealand appeared to have twice as many flower-bearing upright side branches than plants from the native ranges of North America. The allozyme analysis suggested that most of the genetic variation in the native ranges has been introduced to the invasive ranges. This suggests that populations in the invasive ranges have sufficient genetic variability for populations to evolve and adapt to the local selective pressures. It is therefore possible that after introduction to New Zealand, *E. guttata* has continued to evolve and adapt to the unique environmental pressures incurred.

I found there must be an alternative explanation for the high proportion of genetic variation I observed in both common gardens. The results support earlier observations that populations of *E. guttata* can express a variety of phenotypes. When taken in conjunction with the conclusions of chapter 2, these results (Table 3.4) provided further evidence of a genotype by environment (GxE) interaction. This means that different genotypes are responding to different environments in different ways, which increases the number of phenotypes observed across both gardens.

While I observed no obvious latitudinal trend in jdate first bud, of note was that the North Island population (population 35) was the last population to produce buds in both the Ilam and Cass gardens. This makes sense in terms of an adaptation to growing season – we might

expect the North Island population to flower later as summer daylength increases from a high (South) to low (North) latitude. The difference between the production of the first bud by plants at Ilam and plants at Cass was on average 10 days (Figure 3.2) suggesting a GxE interaction and a role of phenotypic plasticity.

Moreover, the Ilam bar chart (Figure 3.2) showed population 23, the most southerly of all my populations (SI_SE), produced first buds on average half a day earlier than the North Island population. The two populations are separated by a latitude of over 10° and are situated in very different climatic zones (NIWA, 2017). The North Island population is situated in a sub-tropical climatic zone and experiences warmer temperatures compared to SI_SE which characteristically experiences cool coastal breezes and low coastal cloud (NIWA, 2017). While this be a latitudinal effect, the fact that I have only one North Island population means that it is impossible to draw firm conclusions. Future work could use more northern populations to definitively confirm or reject the presence of a latitudinal trend in phenology.

I can 100% guarantee that all clones of population 36 are identical (they are all cuttings from a single ramet) and should therefore elicit performance characteristics that are similar, if not identical, among replicates in both common gardens. When identified on a PCA, I could clearly see that the clones from population 36 were behaving differently at both Ilam and Cass (Figure 3.6).

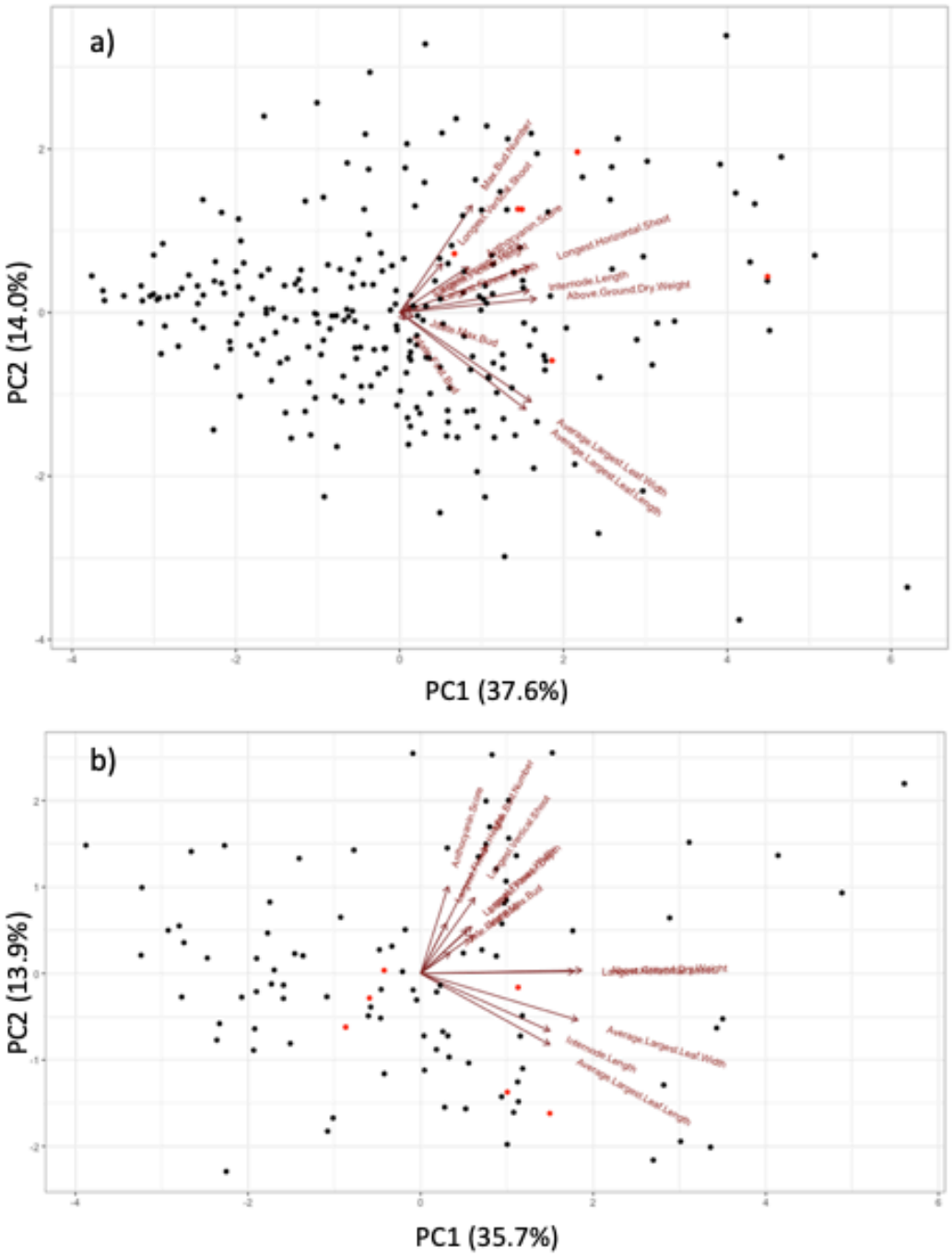

Figure 3. 6: Principal component analysis (PCA) biplots delineating principal component 1 and principal component 2. Each dot represents an individual observation. The red dots represent the individual clones from population 36 specifically. The x-axis represents the first PC and the y-axis represents the second PC. a) PCA for plants grown at Ilam; b) PCA for plants grown at Cass.

Latitudinal differences have been shown in the phenology, morphology, physiology and life history of populations (Phillips *et al.*, 1983; Lowry *et al.*, 2008; van Kleunen & Fischer, 2008; Wu *et al.*, 2010; De Frenne *et al.*, 2011; Lowry *et al.*, 2014; Sheth & Angert, 2014; Qiu *et al.*, 2018). It is therefore interesting that an absence of latitudinal trend was observed in the current study, particularly for phenological traits commonly known to correlate with photoperiod and temperature in a range of plant species (Phillips *et al.,* 1983; De Frenne *et al.*, 2011; Lowry *et al.*, 2014; Qui *et al*., 2018). The lack of correlation between between plant size measures (horizontal and vertical shoot measures) is consistent with the results of an earlier study by van Kleunen and Fischer (2008) who showed plant height of *E. guttata* was not affected by differences in latitude.

Literature on plant latitudinal trends specifically in New Zealand is relatively depauperate. Of the current available research, it appears that latitudinal trends in New Zealand provide mixed findings. Harris (2002) observed a latitudinal trend in the native *Leptospermum scoparium* whereas Rapson and Wilson (1992) did not observe a latitudinal trend in the non-native *Agrostis capillaris*, despite it having been introduced over 130 years ago. *E. guttata* was introduced to New Zealand around approximately the same time as *A. capillaris*. Both species are rhizomatous, occurring as perennials and are deemed invasive in New Zealand habitats. The weak latitudinal trend across New Zealand could suggest that 130 years is not enough time to develop a strong diversity in phenotypes that are linked with latitude in New Zealand. An alternative, and more plausible explanation is that the weak latitudinal trend observed in *E. guttata* could be attributed to its multiple introductions.

The mode of expansion in this species may explain of the weak latitudinal trend I found for *E. guttata*. It is easily spread through anthropogenic translocation of seed and plant fragments or hydrochory (Kiang & Hamrick, 1978; Truscott *et al.*, 2006). These systems facilitate long distance dispersal and promote gene flow between otherwise isolated populations. Combined with the genetic variability (van Kleunen & Fischer, 2008) and phenotypic plasticity identified in New Zealand populations, the easy movement of individuals would prevent populations from evolving genetically distinct adaptations.

It is possible that a microclimatic variable not measured in this experiment was the overriding factor responsible for the observation of phenotypic differences in the field. From this research, we can disregard latitudinal variations as the driving force for phenotypic variation. Correlations between performance traits and the environmental variables measured also came up fruitless. However, several environmental variables were not measured in this study. These include variables such as soil quality, soil composition, light intensity and pH which have been identified as affecting plant performance in *E. guttata* and similar species (Li *et al.*, 1998; Olsson *et al.*, 2002; Willis & Hulme, 2002; Michalski *et al.*, 2017; Qui *et al*., 2018). In addition, many of the environmental variables recorded such as temperatures, were taken from weather station readings. The distance between the sampling location and the nearest weather station fluctuated from between 1 km and 32 km and therefore provide less accurate weather readings.

Finally, New Zealand varies over a latitude of approximately 12.5°. This is minimal when compared to the latitude of North America, which varies in excess of 40°, where the majority of latitudinal research on *E. guttata* is conducted. It is possible that the length of New Zealand does not cover a latitude long enough to evolve latitudinal-diverse phenotypes. It is important to note that while New Zealand does encompass a relatively large latitude compared to other island nations, the majority of New Zealand's exotic species (and those in other oceanic island countries) have been introduced from continental countries (Vitousek *et al.*, 1997; Proches *et al.*, 2008; MacLeod *et al.*, 2009) which encompass much larger latitudes.

3.5 Summary

The aim of this study was to investigate the effect of latitude on populations of *Erythranthe gutatta* from across New Zealand, grown in two geographically separate common gardens. The populations came from 35 locations from seven geographically-distinct regions in New Zealand. Sampling sites were exposed to heterogenous environments which differed in environmental variables including latitude, longitude, rainfall, sunlight hours and temperature. Across all measured traits, I found almost no indication from any of the analyses of a latitudinal trend in plant performance. Internode length was the only performance measure to show a weak, but significant effect of latitude. Phenotypes showed high variability

in their expression among populations, regions and garden locations. The results further highlight the tolerance of *E. guttata* to a range of environmental conditions, and the ability to rapidly alter phenotypic expression to compensate for changes in environment.

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Chapter 4

To what extent do maternal effects influence genetic and plastic responses of first-generation plants in New Zealand populations of *Erythranthe guttata*?

4.1 Introduction

The common garden experiments described in chapter 2 and have shown evidence for strong genetic differences among New Zealand populations as well as the ability of traits to be phenotypically plastic, i.e. individuals express high levels of genetic variation through production of a plethora of phenotypes across a wide range of environments. In addition, the results of chapter 3 provide little evidence for the presence of a latitudinal trend across New Zealand.

A strong body of research emphasizes the effect of genotype, environment and the genotype by environment (GxE) interaction in the variation of individual phenotype. However, increasingly, maternal effects are being included as determinants of phenotypic variation (Weiner *et al.*, 1997). Maternal effects are the contribution of the maternal parent on the phenotype of the offspring (Roach & Wulff, 1987; Weiner *et al.*, 1997; Stjernman & Little, 2011). Studies have shown that maternal effects influence life history traits, for example seed size, germination timing and success, leaf production, and growth rates (Schuler & Orrock, 2012). They have the potential to provide adaptive plasticity across several generations (Galloway, 2005). Maternal effects are especially prominent in the juvenile stages of development e.g. in seed size and germination rate (Stratton, 1989; Montalvo, 1994; Bischoff & Mueller-Schaerer, 2010). Maternal effects have the ability to mask alternative factors affecting plant performance including inbreeding depression and genetic differences (Libby & Jund, 1962; Roach & Wulff, 1987; Montalvo, 1994; Galloway, 1995; Schwaegerle *et al.*, 2000; Galloway, 2005; Latzel & Klimesova, 2010; Dong *et al.*, 2017).

Maternal effects can be separated into genetic and non-genetic effects. Genetic maternal effects are inherited through genetic material contributed solely by the mother such as via the mitochondria and chloroplasts (Platenkamp & Shaw, 1993; Weiner *et al.*, 1997). They arise through genetic differences among mother plants and can result in differences in the allocation of resources to offspring plants through seeds or rhizomes (Weiner *et al.*, 1997; Galloway, 2005). Non-genetic maternal effects emerge when environmental differences influence the mother plants growth, development and subsequent seed provisioning (Platenkamp & Shaw, 1993; Weiner *et al.*, 1997; Mousseau & Fox, 1998) and vegetative propagation (Libby & Jund, 1962; Schwaegerle *et al.*, 2000; Latzel & Klimesova, 2010; Dong *et al.*, 2017). A variety of maternal environmental features affect phenotypic expression in offspring including nutrient levels, photoperiod, light quality, temperature and plant hormones (Stratton, 1989).

Examples of maternal effects in natural systems are ubiquitous. In annual plants, nutrient (e.g. nitrogen, phosphorus, potassium and calcium) concentration has been shown to affect germination rates and biomass (Parrish & Bazzaz, 1985; Galloway, 2001). A deficiency in nutrient contrentation can be detrimental to plant growth while too much can be toxic. Additionally, the availability of water to the maternal plant has a cascade effect on the offspring by affecting seed traits and plant development (Galloway, 1995; Riginos *et al.*, 2007; Sultan *et al.*, 2009; Germain & Gilbert, 2014). Maternal drought has been shown to decrease stomatal conductance of offspring and increase sensitivity to abscisic acid in *Impatiens capensis* (Riginos *et al.*, 2007). Furthermore, intraspecific competition among the maternal plant population has been shown to affect time to germination and seed mass (Stratton, 1989; Platenkamp & Shaw, 1993) as well as maintaining strong maternal effects into adulthood (Galloway, 1995. In the vegetatively reproducing perennial species *Alternanthera philoxeroides*, maternal herbivory effects can influence growth traits in subsequent generations (Dong *et al.*, 2017). These in turn have repercussions on plant growth and reproduction.

Maternal effects from the native range dissipate in plants grown in standardized conditions for several generations. Growing plants for multiple generations under standard conditions account for and remove environmental maternal effects (Libby & Jund, 1962; Galloway &

Fenster, 2000; Santamaria *et al.*, 2003; Bischoff & Mueller-Schaerer, 2010). By using the F2 generation, grown in the same manner as the F1 generation, environmental maternal effects are considerably reduced or dispelled (Libby & Jund, 1962; Bischoff & Mueller-Schaerer, 2010).

4.1.1 Objective three

The aim of this section of my thesis was to determine whether the results and conclusions made from the F1 generation clones (Chapters 2 and 3) were corroborated in the second generation. It was important to investigate whether without (or severely reduced) maternal effects, my conclusions around the contributions of genetics vs phenotypic plasticity in explaining the variation among New Zealand populations of *E. guttata* still held, as well as my conclusions around evidence for latitudinal trends.

My null hypothesis was that there would be no difference between F1 and F2 generations of *E. guttata* populations in New Zealand.

4.2 Methods

4.2.1 Experimental Background

Exotic plant species often elicit patterns of expansion when colonising and naturalising in a novel range. These patterns are controlled by the contribution of genetic variability and/or phenotypic plasticity in the invading population. Common garden experiments are traditionally utilised in testing the presence of genetic variability and/or phenotypic plasticity in populations. This method involves collecting samples from various populations, often those which vary in environmental conditions, and relocating them to a region with standardized conditions, known as a 'common garden'. The common garden, and all individuals grown within it, experience growing conditions that are as identical as possible (e.g. light, water, nutrients and temperature).

4.2.2 Population locations and sampling

I used the same populations of *Erythranthe guttata* and the same sampling methods described in Chapter 2 (Table 2.2) for first generation (F1) individuals.

During the autumn of 2018, tip cuttings of a consistent size from each of the 366 individual plants (Figure 4.1) were made to propagate a population of a second (F2) generation population. These were grown in a cool glasshouse through the winter. I trimmed the cuttings regularly to make sure they remained small plants during the winter months so as that plants would be of a consistent size when I planted them into the common garden. I moved the plants to the common garden on September 4, 2018.

Figure 4. 1: Tip cuttings for F2 generation propagation.

4.2.3 Common garden set up

I used the same common garden set up as described in Chapter 2 (Table 2.4) for the 2018-2019 year. In the second year, because of time constraints I focused on only the Ilam garden. I included all of the 366 plants, held in pots and arranged in a series of rows (Figure 4.2). I established this garden on September 3, 2018.

Figure 4. 2: The Ilam garden layout on September 3, 2018. This image was taken by a drone (Photo credit: C. Antony).

4.2.4 Performance measurements

To estimate the genetic component of the variation among the 35 populations (clones) after a year in the common garden (i.e. without maternal effects). I measured 15 performance traits (Table 4.1).

Table 4. 1: The performance traits measured for Erythranthe guttata *for the summer of 2018-2019. New traits are represented by 'N' and traits re-measured are represented by 'R'.*

N vs R	Performance Measure	Unit of measurement
N	Largest leaf surface area	mm^2
N	Leaf shape	leaf length:leaf width ratio
N	Date of first flower	Julian date
N	Date of maximum flower	Julian date
N	Maximum flower number	
N	Petiole length	mm
R	Largest leaf length (Figure 2.6)	mm
R	Largest leaf width (Figure 2.6)	mm
R	Longest horizontal shoot	mm
R	Longest vertical shoot	mm
R	Internode Length (measured between the second and third internodes from the tip)	mm
R	Date of first bud	Julian date
R	Largest flower height (Figure 2.6)	mm
R	Largest flower depth (Figure 2.6)	mm
R	Largest flower width (Figure 2.6)	mm

I added an additional six measures (indicated in Table 4.1) to my previous year's performance traits: during the year I had become aware of several relevant investigations in the invasion biology literature which had demonstrated variation in these traits between native and invasive plant populations (Kollmann & Banuelos, 2004; Bell & Galloway, 2008; Lowry *et al*., 2008; Weijschede *et al*., 2008; Williams *et al*., 2008; Murren *et al*., 2009; Wu *et al*., 2010; Ebeling *et al*., 2011; Frei *et al*., 2012; Hamann *et al*., 2017; Groot *et al*., 2018). Time constrictions meant that it was not feasible for me to re-measure plant dry weight, instead, I used leaf and shoot measurements as a non-destructive method for quantifying plant size (Groot *et al*., 2018). Internode length is indicative of the speed of growth. Plants that grow quickly tend to have larger distances between internodes than plants that grow slower. I measured internode length as the distance between the second and third internode from the

tip of the longest shoot (either horizontal or vertical) (Figure 2.7b). I measured the date to first bud, date to first flower, date to maximum flower number and the maximum number of flowers as these are representative of an individual plants reproductive output (Murren *et al*., 2009; Ebeling *et al*., 2011).

In the analysis, I used the maximum trait value recorded for each individual over the entire study period (September 4, 2018-December 17, 2018) for flower height, depth, width and flower number. I recorded leaf length, width and surface area from the largest observed leaf on December 17, 2018. I took all other morphological measures within the week prior to harvesting. I collected floral measures and bud numbers every two or three days between October 31, 2018 until December 10, 2018. I quantified the date of first flower by recording the date that the first flower completely opened (Williams *et al*., 2008).

4.2.5 Analysis

In the methods, I explained that at each site (population) I collected one plant. As plants tend to spread vegetatively (Figures 2.2a to 2.2c) rather than a single stem, I collected a bunch of closely intertwined stems (Figures 2.2c and 2.2d). I assumed these were clonally connected and therefore genetically the same (except for random somatic mutations). My tip and internode cuttings came from across the bunch. In retrospect, this method of collecting was potentially flawed as more than one genetic clone may have been growing together. If this was the case, it would mean that my tip cuttings from a 'single clone' may in fact be from more than one clone. Overall, there was very little evidence to suggest this was the case – except for in three populations (3, 15 and 26) where some 'clones' out of my clonal replicates appeared to be phenotypically different from the rest (Figure 4.4). In population 3, from Taylor River, Blenheim, I found three quite distinct morphologies among my clones (Figure 4.4a). In population 26, from the Tokanui-Gorge Road Highway, Fortrose, and population 15, from Omarama-Lindis Pass Road, Waitaki, I found two distinct morphologies among my clones (Figure 4.4b and 4.4c respectively).

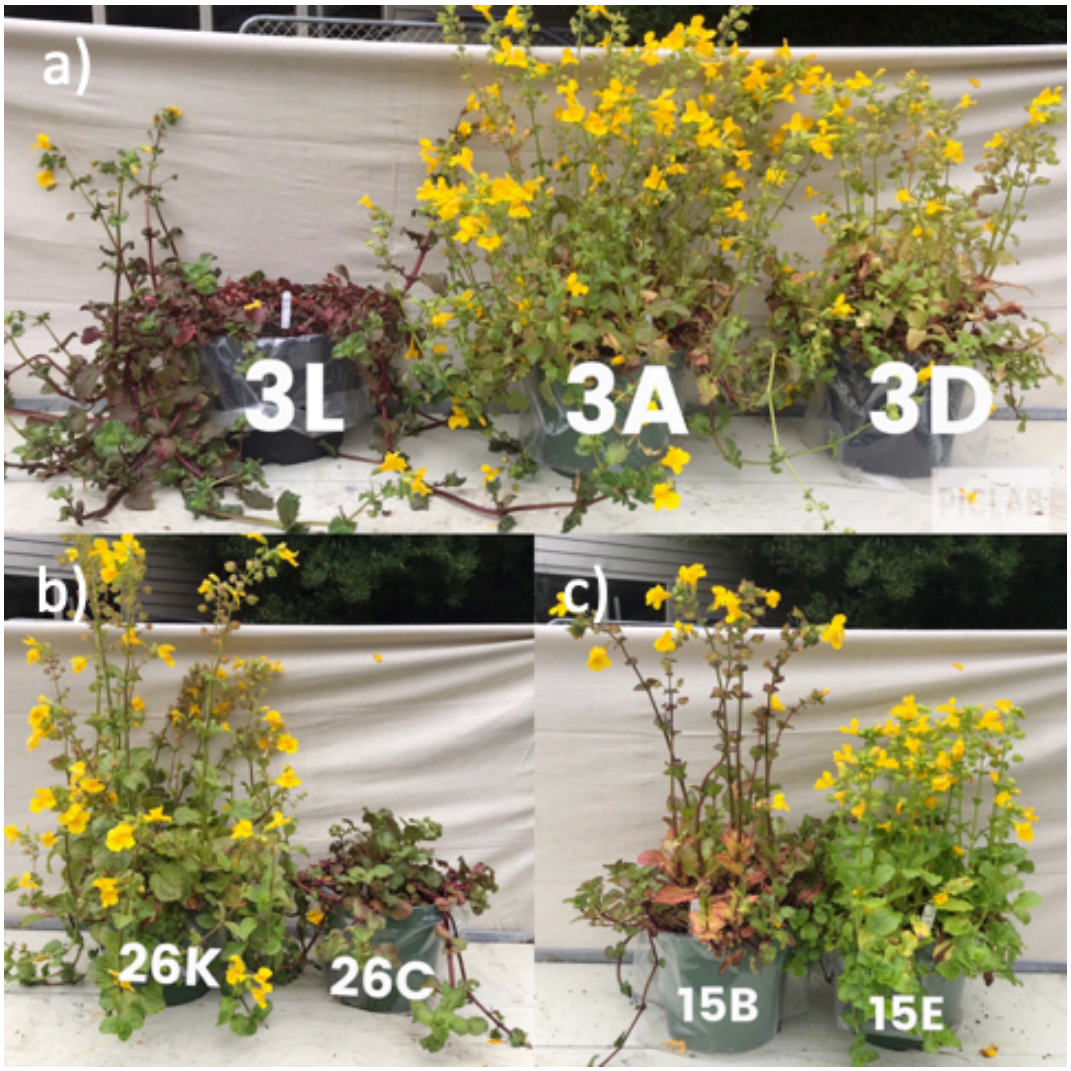

Figure 4. 3: Images of the different ecotypes within three different populations: a) shows population 3, where individual '3D' represents the common phenotype. Individuals '3A' and '3L' represent the uncommon phenotypes; b) shows population 26, where individual '26C' represents the common phenotype and individual '26K' the uncommon phenotype; c) shows population 15, where individual '15E' is the common phenotype and individual '15B' the uncommon phenotype.

Including the within-population variation of the 'odd' individuals would severely disrupt my analyses therefore I removed the few 'different' morphotypes from the analysis. This meant that for all 35 populations I had one genetic clone (to the extent that morphological observations suggested in a common garden). Specifically, I removed the following individuals: 3A, 3H, 3L, 15B, 26H and 26K. From then on, I assumed that all plants from a single population were of the same vegetative clone.

I first created histograms to visually assess patterns of trait distribution among the clones of all populations. The statistical distributions were all normally distributed except for largest leaf surface area, petiole length and maximum flower number. This was further evidenced by a normal Q-Q plot and a residuals vs fitted plot. These three trait values required a log transformation to satisfy the assumptions of homoscedasticity and normality for the analyses.

I used a principal component analyses (PCA) as a preliminary analysis to visualize and patterns among populations which may be due to latitudinal trends. A PCA compresses multi-dimensional data, in this case, multiple performance measures, into a small dataset that can be visualized in two-dimensions. The axes are represented by principal components (PCs), values given to a set of variables which were linearly uncorrelated. Each individual on a biplot was represented by a dot that represents the position of each 'clone' in relation to the first two PCs. The biplot also illustrates how the performance traits map onto the first two PCs. On the biplot they are vectors (arrows) originating from the centre and pointing in the direction in which high values of a trait move. I ran the PCA using all 15 performance measures (Table 4.1).

From looking at the distribution of all the 15 performance measures along the first two axes of the PCA, I was able to see which of the performance traits were correlated (i.e. which traits headed in the same direction). I identified six uncorrelated performance measures (i.e. traits with distinct vectors) (Table 4.2). For the remainder of the analyses, these six performance measures will be the only ones presented in this chapter, unless stated otherwise.

Table 4. 2: Six uncorrelated performance measures as detected from the PCA biplot of the first two PCs.

Performance Trait
Largest leaf surface area
Leaf shape
Internode length
Jdate first bud
Max flower number
Flower depth

To test for genetic differences among populations, I used an analysis of variance (ANOVA) on each of the 15 performance measures individually among all of the populations. The ANOVA indicated whether differences were genetic or plastic.

I quantified 'leaf shape' by calculating the leaf length to leaf width ratio (Frei *et al.*, 2012; De Carvalho *et al*., 2017). I used the ANOVA output testing for differences among populations in leaf shape, in order to highlight the lack of tight association between trait values and region. To do this, I produced a bar chart of each populations average leaf shape, ranked from smallest to the largest.

To determine to what extent any of the variation among the populations was due to latitude and region, and their interaction, I used a two-way analysis of covariance (ANCOVA). The model included the region, latitude and the interation effect, region*latitude. The primary use of a two-way ANCOVA was to understand if there was an interaction between region and latitude on the leaf surface area, leaf shape, internode length, jdate first bud, maximum flower number and flower depth.

I compared the results of this chapter to the results from chapters 2 and 3. This determined if losing maternal effects lead to similar or different results.

Full statistical analyses, using all 15 performance measures can be found in the appendix (Appendix H).

All statistical analyses were undertaken using R statistical software version 3.5.1 (R Core Team, 2013).

4.3 Results

I found evidence that maternal effects had masked genetic differentiation, phenotypic plasticity and latitudinal trends in the F1 generation. In particular, genetic differences and latitudinal trends became far more prominent with the dissipation of maternal effects.

The first principal component (PC1) accounted for 28.3% of the total variance (Table 4.3) and PC2 accounted for 18.1% of the total variance. Together, PC1 and PC2 accounted for 46.4% of the total variation. This means that the traits with the longest vectors (jdate first bud and horizontal shoot) along the first two PCs likely accounted for a large proportion of the variation. Cumulatively, PC1 through to PC4 explained 66.5% of the variation.

Table 4. 3: Principal component analysis (PCA) table for the explained variation among regions across 15 performance measures. "PC" represents principal component.

	PC1	PC2	PC3	PC4
StdDev	1.852	1.482	1.163	1.036
Proportion of Variance	0.283	0.181	0.112	0.089
Cumulative Proportion	0.283	0.464	0.576	0.665

As in the F1 generation, the principal component analysis (PCA) biplot of the first two PCs illustrated that no two 'clones' expressed the exact same phenotype across all 15 performance measures (Figure 4.4) and again, there was a diffuse spread of clones with a tendency for clones from the SI_C (orange dots on the figure) lying towards the left of the biplot – suggesting a difference in leaf shape. SI_NE were towards the right.

From looking at the vectors of the performance measures (Figure 4.4), clones with longer horizontal shoots and larger leaves may lie towards the right of the horizontal axis. Plants with a larger leaf shape (leaf length:leaf width ratio) may lie towards the bottom left of the PCA. In contrast, tall clones with long, vertical shoots and many large flowers may lie towards the bottom of the PCA – low down on PC2. Early flowering clones may be clustered towards the centre of the PCA, with later flowering individuals found towards the top of PC2. Flower height, longest vertical shoot and jdate first bud contribute most to the second axis.

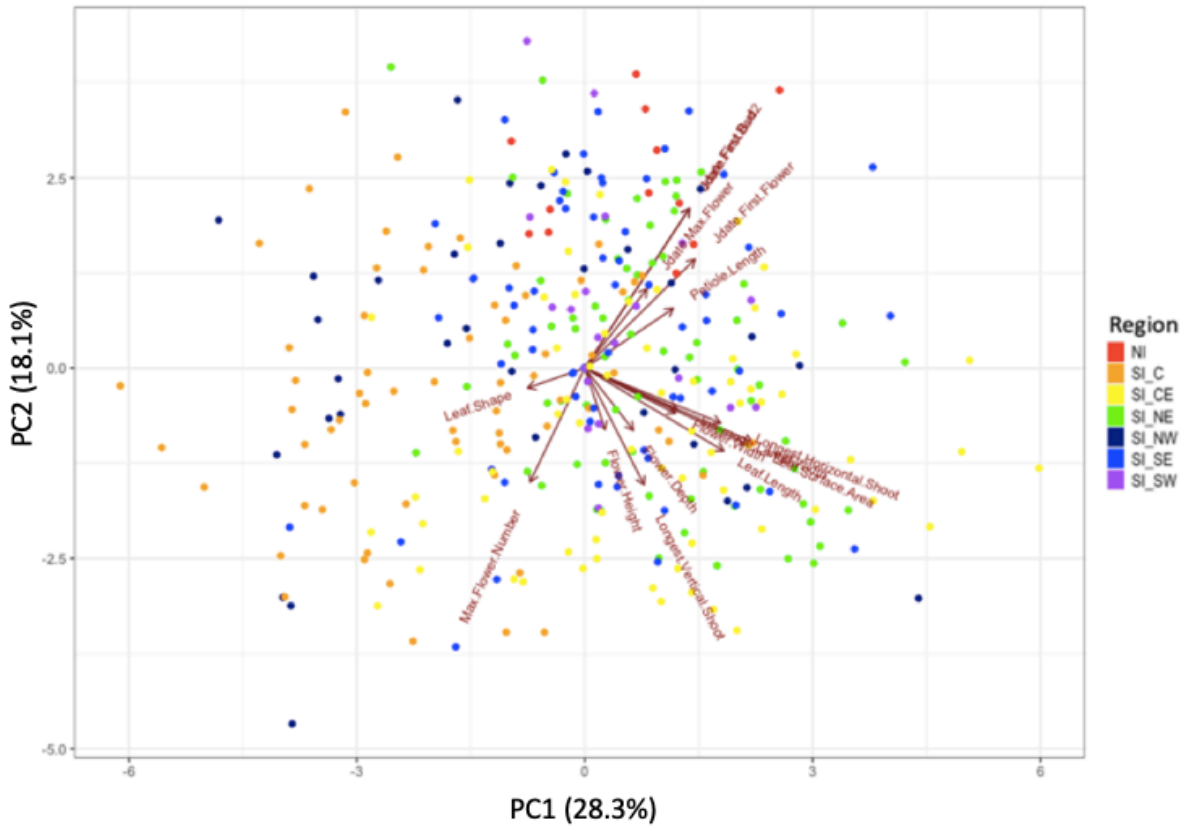

Figure 4. 4: *Principal component analysis (PCA) biplot displaying the first principal component (x-axis) and the second principal component (y-axis). Regions have been colour-coded; North Island (NI), South Island Central (SI_C), South Island Central East (SI_CE), South Island North East (SI_NE), South Island North West (SI_NW), South Island South East (SI_SE), South Island South West (SI_SW).*

I carried out an analysis of variance (ANOVA) on each of the populations. The results of the ANOVA on each of the 15 performance measures across all 35 populations indicated a

significant ($p < 0.05$) difference in all performance traits in at least one of the populations (Table 4.4). These results corroborate the conclusions made in chapter 2. However, in contrast to my findings from chapter 2, without maternal effects, all performance measures were significant (Table 4.4).

An example of this variation can be illustrated through a boxplot for internode length (Figure 4.5).

Table 4. 4: Analysis of variance for differences in performance measures among populations.

**Significant ($p < 0.05$)*

Performance Measure	Ilam		
	F value	df	p value
Largest Leaf Surface Area	6.6	278	**0***
Largest Leaf Length	5.25	276	**0***
Largest Leaf Width	6.89	276	**0***
Leaf Shape	5.19	276	**0***
Longest Horizontal Shoot	8.19	277	**0***
Longest Vertical Shoot	7.06	277	**0***
Internode Length	2.31	277	**0***
Jdate First Bud	12.47	290	**0***
Jdate First Flower	9.39	249	**0***
Jdate Max Flower	2.96	241	**0***
Max Flower Number	7.52	239	**0***
Petiole Length	13.09	228	**0***
Largest Flower Height	3.83	226	**0***
Largest Flower Depth	3.68	226	**0***
Largest Flower Width	6.19	226	**0***

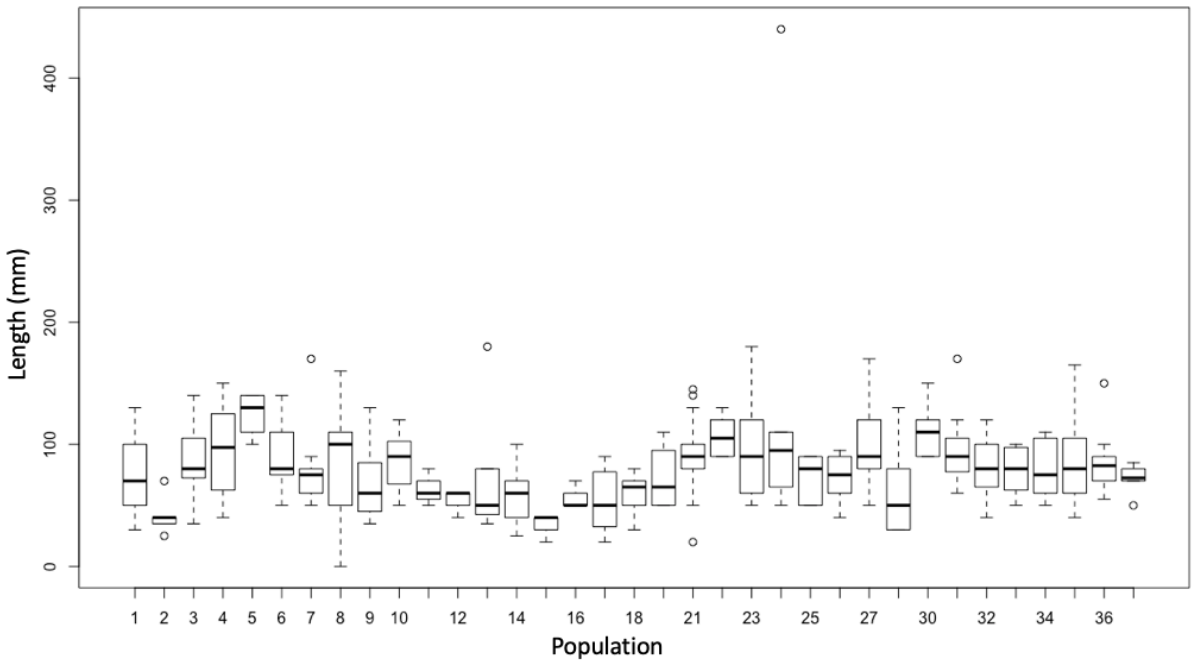

Figure 4. 5: Boxplot of the internode length for all of the populations grown at Ilam for the summer of 2018-2019.

The bar chart based on ANOVA, ranked leaf shape bar chart failed to show clustering of populations by region (Figure 4.6). There was a ratio difference of 1.8 between the smallest ratio and the largest ratio (Figure 4.6, 4.7 and 4.8). Population 5 from SI_NE had the smallest leaf shape (2.76) while population 12 from SI_C had the largest leaf shape (4.6) (Figure 4.8). While most of the populations from SI_C were ranked with the top 50% of populations for leaf shape, population 14 had a much smaller leaf shape (3.27).

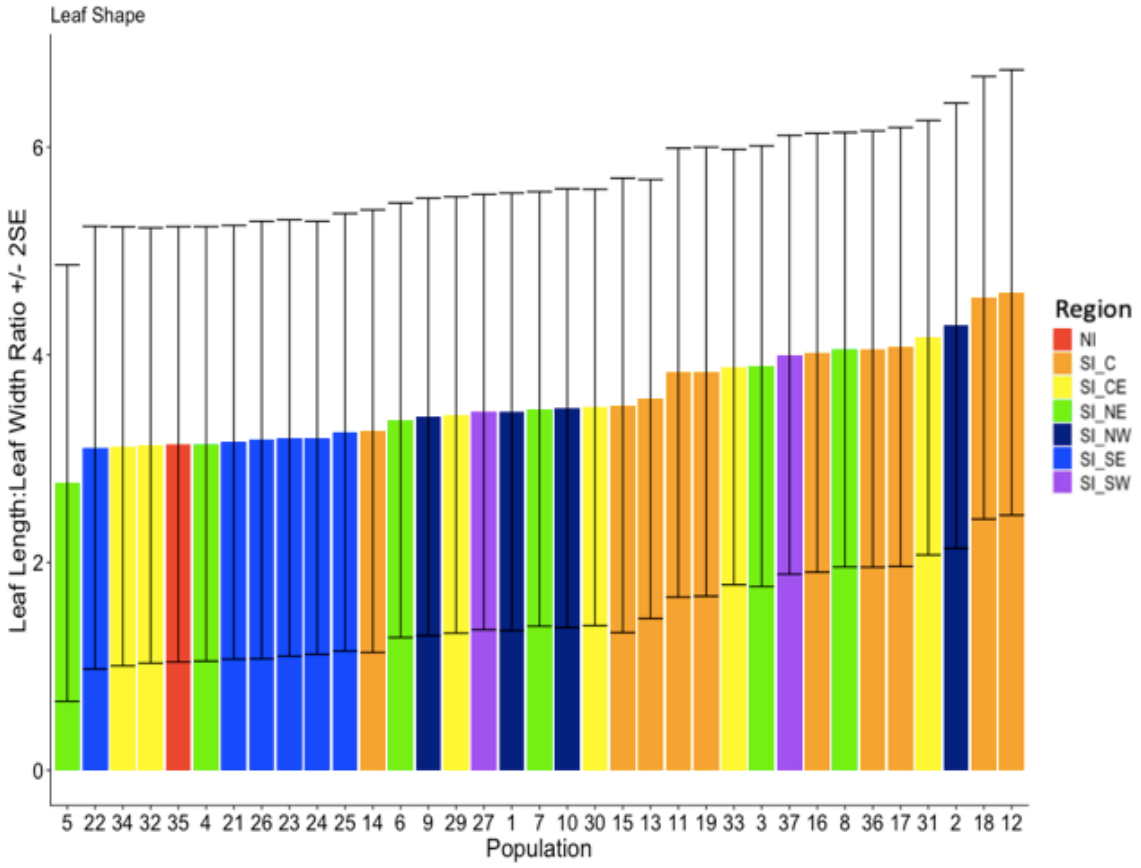

Figure 4. 6: The average leaf shape (leaf length:leaf width ratio) for each population between September 1, 2018 and December 10, 2018. The regions are colour coded; North Island (NI), South Island Central (SI_C), South Island Central East (SI_CE), South Island North East (SI_NE), South Island North West (SI_NW), South Island South East (SI_SE), South Island South West (SI_SW). The y-axis represents the leaf length:leaf width ratio +/- 2 standard error (SE) and along the x-axis are the populations.

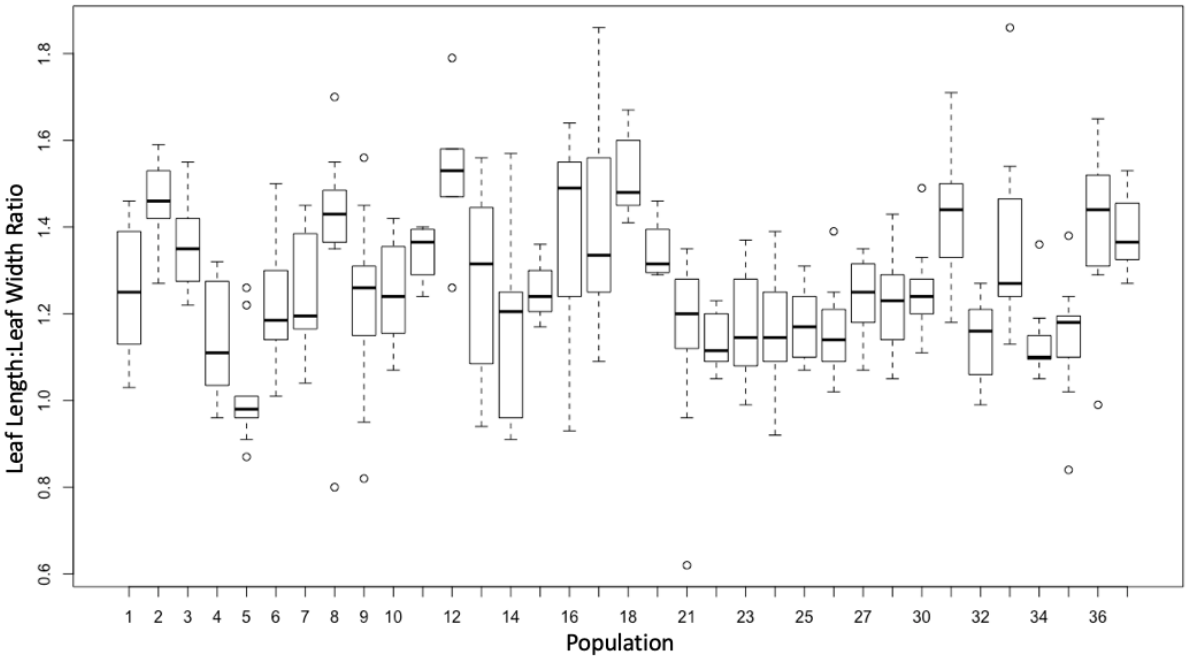

Figure 4. 7: Boxplot of the leaf shape for all of the populations grown at Ilam for the summer of 2018-2019.

*Figure 4. 8: Two E*rythranthe guttata *leaves. The leaf from population 5 (left) had the smallest leaf shape ratio and the leaf from population 12 (right) had the largest leaf shape ratio.*

The two-way analysis of covariance (ANCOVA) was the only analysis to suggest any latitudinal trend. On its own, I found a significant ($p < 0.05$) interaction between latitude and five of the

six performance measures presented (Table 4.5). The trait showing no interaction with latitude was flower depth.

For region alone, I found a significant ($p < 0.05$) effect was found for all six of the performance measures presented (Table 4.5). For flower depth, region alone accounted for 23% of the total variance.

The ANCOVA showed a significant ($p < 0.05$) two-way interaction between region and latitude for leaf surface area, leaf shape, internode length and max flower number.

Table 4. 5: Two-way analysis of covariance of region and latitude showing only six performance measures. Degrees of freedom for the residual is 300, 298, 299, 312, 300 and 248 for log largest leaf surface area, leaf shape, internode length, jdate first bud, log max flower number and flower depth, respectively.

**Significant ($p < 0.05$)*

Performance Measure		**Source of Variation**		
		Region	Latitude	Region*Latitude
Log Largest Leaf Surface Area	F-statistic	13.31	4.62	4.70
	p-value	**<0***	**0.03***	**≤0***
	% Var.	20	1	6
Leaf Shape	F-statistic	9.23	5.60	2.82
	p-value	**<0***	**0.02***	**0.02***
	% Var.	15	2	4
Internode Length	F-statistic	4.84	5.32	2.45
	p-value	**<0***	**0.02***	**0.03***
	% Var.	8	2	4
Jdate First Bud	F-statistic	10.51	20.86	1.85
	p-value	**<0***	**<0***	0.10
	% Var.	16	5	2
Log Max Flower Number	F-statistic	3.91	11.40	4.35
	p-value	**≤0***	**≤0***	**≤0***
	% Var.	7	4	7
Flower Depth	F-statistic	12.18	0.11	0.47
	p-value	**<0***	0.74	0.80
	% Var.	23	0	1

4.4 Discussion

The primary goal of my common garden experiment was to determine if losing maternal effects in 35 New Zealand populations of *Erythranthe guttata* would produce results similar

to those seen in chapters 2 and 3. I achieved this through propagation of an F2 generation under standardized-conditions at the end of the 2017/2018 season. By replicating my Ilam common garden for a following year, I was able to gain stronger evidence for the presence of genetic diversity, phenotypic plasticity and a cline among my 35 populations of *E. guttata*.

It was important that I re-ran the common garden experiment so as to determine the effect maternal influences had on the F1 generation. When comparing my results from this chapter to those of chapter 2 and 3, it was clear to see that maternal effects were masking some of the phenotypic differences between populations. Previously there was very little evidence to suggest a latitudinal trend (Table 3.4) whereas the following year provided more evidence for a cline. In addition, the first year results found evidence for genetic diversity and phenotypic plasticity across some performance measures (Table 2.6) whereas the following year found genetic diversity across all performance measures (Table 4.4). Overall, by collecting data for another season, I was able to show that maternal influences effect the expressed phenotype of *E. guttata* and a reduction in these influences leads to more a prominent latitudinal trend and stronger genetic differences.

Preceding studies have emphasized the influence of maternal effects on phenotypic expression in plants (Roach & Wulff, 1987; Weiner *et al.*, 1997; Stratton, 1989; Montalvo, 1994; Bischoff & Mueller-Schaerer, 2010). Maternal effects can influence an array of performance traits including those which vary in phenology, morphology, physiology and life-history. Maternal experiences which affect subsequent generations include nutrient concentration (Parrish & Bazzaz, 1985; Galloway, 2001), water availability (Riginos *et al.*, 2007; Sultan *et al.*, 2009; Germain & Gilbert, 2014) and competition (Stratton, 1989; Platenkamp & Shaw, 1993; Galloway, 1995).

It is not surprising that I found evidence for large amounts of genetic diversity among my 35 populations. Because of its use in horticulture, *E. guttata* has been introduced to New Zealand multiple times (van Kleunen & Fischer, 2008). Consequently, it has been suggested that most, if not all, of the genetic diversity in its native range of North America has now been introduced to its invasive ranges of Scotland and New Zealand (van Kleunen & Fischer, 2008).

The results of chapter 3 were able to conclude little evidence for latitudinal trends in New Zealand populations of *E. guttata*. As in chapter 3, a PCA biplot (Figure 4.4) failed to show clustering of populations by region (used as a loose proxy for latitude). In addition, the bar chart for leaf shape (Figure 4.6) from the ANOVA (Table 4.4) did not show an observable trend across regions. Nevertheless, an effect of latitude was observed in the two-way ANCOVA (Table 4.5). These results corrobated with the conclusions drawn in chapter 3, however the results from the F2 populations show a much stronger latitudinal trend, i.e. a latitudinal trend across almost all performance measures presented.

The lack of interaction with latitude for flower depth could suggest that flower traits are under strong genetic control and therefore unlikely to change. This may be an avenue for future research.

Evidence for latitudinal trends in New Zealand plant species is mixed. Harris (2002) found the presence of a latitudinal trend in the native species *Leptospermum scoparium* whereas Rapson and Wilson (1992) failed to identify a latitudinal trend in the exotic species *Agrostis capillaris*.

In the F1 generation PCA (Figure 3.6), PC1 accounted for 37.6% (Ilam) and 35.7% (Cass) of the total variance. In the F2 generation PCA (Figure 4.4) the variance for PC1 was weaker and accounted for 28.3% of the total variance. Interestingly, the reverse was noted for PC2 – i.e. PC2 was stronger in Figure 4.4 and accounted for 18.1% of the total variance whereas is Figure 3.6 it accounted for 14% (Ilam) and 13.9% (Cass).

In the common garden, it was interesting to observe that a few individuals within three populations expressed phenotypes different to the norm for their population (Figure 4.3). This was limited to six individuals across three populations. Because the analyses were based on the assumption that all plants from one population were genetic clones, including these individuals in the analysis could potentially lead to misleading results. I therefore removed them from the analysis. Despite removing these 'odd' individuals with a clearly different phenotype, the results still concurred the conclusions made in chapter 2 – the presence of large amounts of genetic diversity and plastic phenotypes.

Of note is that my 'clones' likely represented only a fraction of the variation that may exist in each of the populations.

Figure 4. 9: The common morphotype among all populations in the Ilam garden 2018/2019.

Among all of the populations in the common garden, the majority of *E. guttata* plants expressed a uniform morphotype (Figure 4.9). From the PCA biplot (Figure 4.4) I could not identify any obvious morphotypes – no two morphotypes are the same. At a closer inspection, there are more minute differences in the plant phenotype. For example, large variations in the anthocyanin markings on the flowers have been observed (Figure 4.10) (Millar, *unpublished*). These differences were observed both among river systems and among sites along a single river system (Millar, *unpublished*). Millar also observed that the variability in anthocyanin markings did not increase downstream, which would be expected if *E. guttata* was dispersing downstream.

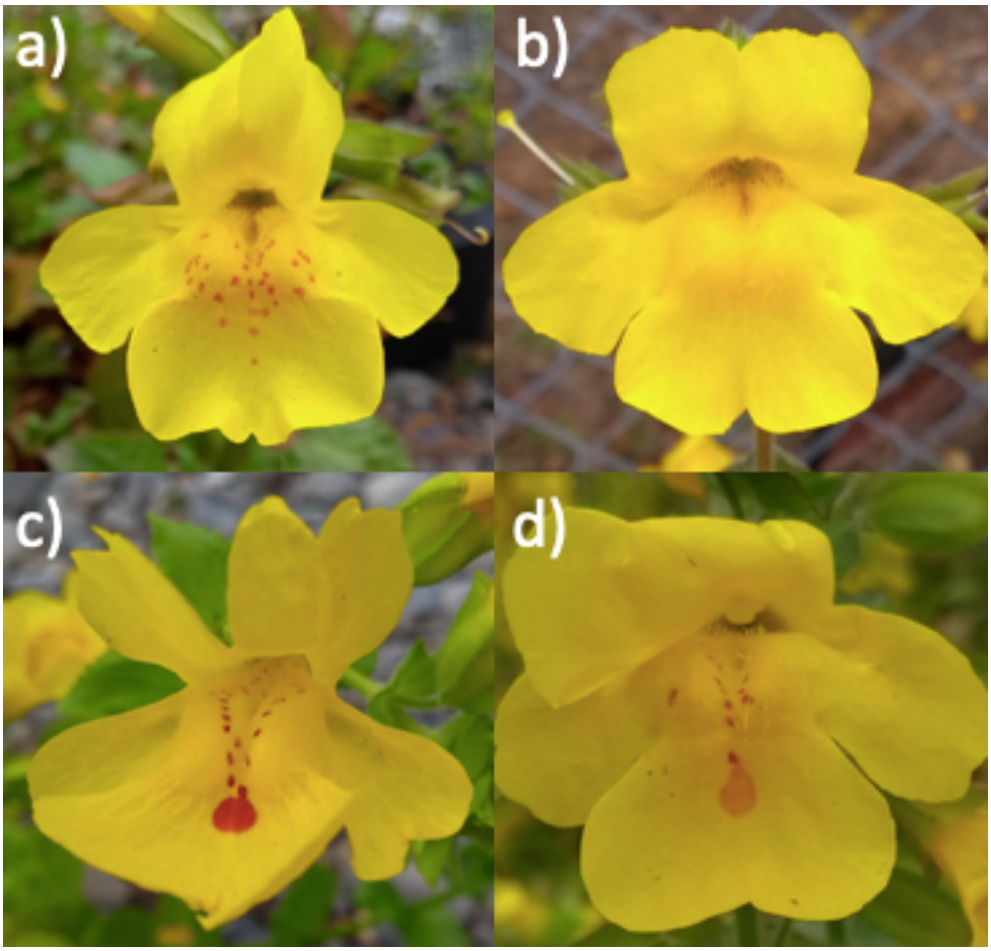

Figure 4. 10: Differences in the anthocyanin markings on four Erythranthe guttata *flowers (Photo credit: A. Millar).*

4.5 Summary

Maternal effects can have significant effects on the expression of plant phenotype. This study aimed to investigate if the loss of maternal effects concurred the conclusions made in chapters 2 and 3 – the presence and co-occurrence of genetic variability and phenotypic plasticity and the presence of a weak latitudinal trend in *Erythranthe guttata*. The F2 generation of 35 populations representing 7 regions from across New Zealand were grown in a common garden experiment. The results have shown that maternal effects play a role in phenotypic expression of *E. guttata* in New Zealand. By minimizing maternal influences, plants showed more prominent genetic differences among populations and a stronger latitudinal trend in phenotype differences could be observed.

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Chapter 5

General discussion and conclusion

5.1 Untangling the sources of phenotypic variation in New Zealand populations of *Erythranthe guttata*

Populations of *Erythranthe guttata* show marked phenotypic differences in heterogenous environments (Hall & Willis, 2006; Lowry *et al.*, 2008; Murren *et al.*, 2009; Lowry *et al.*, 2012; Murren & Dudash, 2012; Peterson *et al.*, 2016). I conducted a common garden experiment to determine whether the phenotypic variation observed in the field was the result of genetic differentiation from natural selection, or phenotypic plasticity. The populations in my common garden were sampled from 35 geographically-separate populations within New Zealand. These populations comprised a variety of habitats including those which differed in terms of latitude, altitude, rainfall, sunlight hours and temperature. To determine which mechanism (genetic differentiation or phenotypic plasticity) was responsible for the observed phenotypic variation in *E. guttata*, I recorded the plant performance of each individual over the summer of 2017/2018. The plant performance measures I evaluated were:

- Above ground biomass (g)
- Largest flower height (mm)
- Largest flower depth (mm)
- Largest flower width (mm)
- Largest leaf length (mm)
- Largest leaf width (mm)
- Longest vertical shoot (mm)
- Longest horizontal shoot (mm)
- Internode Length (mm)
- Anthocyanin concentration
- Maximum bud number
- Date of first bud (Julian date)
- Date of maximum bud number (Julian date)

These phenotypic traits have been shown to elicit defined variation among different localities inhabited by *E. guttata* and similar species in the field (Hall & Willis, 2006; Lowry *et al.*, 2008; Murren *et al.*, 2009; Lowry *et al.*, 2012; Murren & Dudash, 2012; Peterson *et al.*, 2016). If individuals of *E. guttata* from different environments lose their phenotypic differences in a common garden, then these differences are likely to be plastic. Conversely, if phenotypic differences are maintained in a common garden, then these differences must be genetically controlled.

I found statistically significant differences for plant performance traits among gardens and among populations. This was indicative of genetic differentiation in the New Zealand populations of *Erythranthe guttata*. However, I could not find a statistically significant difference for all performance measures among gardens and populations, suggesting the influence of phenotypic plasticity. Overall, my results showed that genotype had the strongest influence on phenotype, however a genotype by environment (GxE) interaction was found for four performance traits, suggesting a dominant effect of phenotypic plasticity on these.

Research suggests genetic differentiation and phenotypic plasticity are mutally beneficial (Pigliucci, 2007). Phenotypic plasticity has even been suggested to facilitate genetic differentiation by providing the initial phenotypic adaptations to overcome a mismatch between phenotype and environment (Sexton *et al*., 2002; Bennington *et al.*, 2012). Plastic responses enable the maximal success of the founding population by changing phenotypic expression in environments where local adaptation has not yet occurred (Pigliucci *et al.*, 1995; Bossdorf *et al.*, 2005; Miner *et al.*, 2005; Hulme, 2008; Palacio-Lopez *et al.*, 2015; Liao *et al.*, 2016; Hamann *et al.*, 2017). In essence, it establishes a 'general-purpose' genotype which is capable of rapidly adapting to disparate environmental conditions (Bossdorf *et al.*, 2005; Hulme, 2008). At the same time, natural selection is leading to genetic sorting of the founding population, albeit at a slower rate than phenotypic plasticity can respond. Natural selection uses existing genetic variation to establish a population that is best adapted to the novel environment through evolutionary adaptation (Lockwood *et al.*, 2005; Miller *et al.*, 2015). Both mechanisms are important to the invasion process as they encourage population estalishment and facilitate expansion processes.

5.2 Evidence for latitudinal trends across New Zealand populations of *Erythranthe guttata*?

The objective of chapter 3 was to investigate whether populations of *Erythranthe gutatta* from across New Zealand, show clinal variation associated with a latitudinal gradient. Studies have demonstrated that environmental differences along a latitudinal gradient (such as temperature, solar radiation, water availability, altitude and photoperiod) pre-empt observable differences in plant growth and reproduction (McMillan, 1967; Li *et al*., 1998; Olsson & Agren, 2002; Willis & Hulme, 2002; Griffith & Watson, 2005; van Kleunen & Fischer, 2008; Bull-Herenu & Arroyo, 2009; Michalski *et al.*, 2017; Qiu *et al*., 2018). I used the same 35 populations and 13 performance measures from chapter 2 in this study.

Across the 13 performance traits I studied, I found little evidence to suggest the presence of a latitudinal trend across New Zealand. I conducted a principal component analysis (PCA) biplot and created bar charts using the analysis of covariance (ANOVA) results (from chapter 2) showed no clustering of populations by region. Region was used as a loose proxy for latitude. In addition, the performance measures did not strongly correlate with any of the environmental variables I recorded. However, the analysis of covariance (ANCOVA) was the only analysis to suggest the presence, albeit weak, of a latitudinal trend in internode length. I was unable to determine with certainty the direction of this trend.

The analyses showed there was large variability in phenotypic expression of performance measures, no two 'clones' are expressed the exact same phenotype. This suggested there was a high tolerance to wide spatial heterogeneity in the 35 *E. guttata* populations. Since internode length was the only trait found to present a latitudinal trend, it may be possible that the lack of latitudinal trend for other traits may be due to another environmental feature, not measured in this study, or the consequence of multiple introductions.

Latitudinal trends in *E. guttata* have been identified in previous studies. For example, van Kleunen and Fischer (2008) identified a latitudinal trend in vegetative reproduction and Wu *et al.* (2010) commented on the phenotypic variation due in part to the water availability along a latitudinal gradient (increased precipitation and decreased evapotranspiration with

increasing latitude) (Truscott *et al.*, 2006; Bull-Herenu & Arroyo, 2009). However, latitudinal trends in New Zealand plant species provide mixed results (Rapson & Wilson, 1992; Harris, 2002). Harris (2002) found evidence for a latitudinal trend in native *Leptospermum scoparium* whereas Rapson and Wilson (1992) failed to find evidence for a latitudinal trend in the exotic *Agrostis capillaris*. Overall, the weak evidence for a latitudinal trend in *E. guttata* may be an artefact of multiple introductions (van Kleunen & Fischer, 2008). Alternatively, it is possible, although less likely, that *E. guttata* has had enough time to succumb to genetic sorting by latitudinally-controlled environmental pressures.

5.3 To what extent do maternal effects influence genetic and plastic responses of first-generation plants in New Zealand populations of *Erythranthe guttata*?

Over a broad spatial scale, populations of *Erythranthe guttata* show obvious phenotypic differences (Hall & Willis, 2006; Lowry *et al.*, 2008; Murren *et al.*, 2009; Lowry *et al.*, 2012; Murren & Dudash, 2012; Peterson *et al.*, 2016). I replicated my common garden experiment to determine whether removal (or a severe reduction) of maternal influences still produced the same results as chapters 2 and 3 – presence of genetic differentiation, phenotypic plasticity and little evidence for a latitudinal trend. I used the same clonally propagated populations from chapters 2 and 3 (5.1 and 5.2) in this study however, unlike the earlier chapters, the plants I used here were second generation individuals. To determine whether the conclusions made in chapters 2 and 3 (5.1 and 5.2) were affected by maternal effects, I propagated the second generation of plants and planted them into a single common garden. I re-recorded nine of the same plant performance measures from the previous year, as well as including six new performance measures. The plant performance measures I recorded were:

- Largest flower height (mm)
- Largest flower depth (mm)
- Largest flower width (mm)
- Petiole length (mm)
- Largest leaf length (mm)
- Largest leaf width (mm)
- Largest leaf surface area (mm^2)

- Leaf shape (leaf length:leaf width ratio)
- Longest vertical shoot (mm)
- Longest horizontal shoot (mm)
- Internode Length (mm)
- Date of first bud (Julian date)
- Date of first flower (Julian date)
- Date of maximum flower (Julian date)
- Maximum flower number

As outlined earlier, these phenotypic traits show distinct variation among the different habitats occupied by *E. guttata* and related species in the field (Kollmann & Banuelos, 2004; Bell & Galloway, 2008; Lowry *et al*., 2008; Weijschede *et al*., 2008; Williams *et al*., 2008; Murren *et al*., 2009; Wu *et al*., 2010; Ebeling *et al*., 2011; Frei *et al*., 2012; Hamann *et al*., 2017; Groot *et al*., 2018).

I found significant differences in all 15 performance measures among populations, indicative of genetic differentiation. In addition, I found stronger evidence for a latitudinal trend. However, as with the conclusions made in Chapter 3 the presence of latitudinal trends could only be found through an analysis of covariance (ANCOVA). I can therefore conclude that maternal effects are having a prominent effect on phenotypic expression in the 35 populations of *E. guttata*.The reduction of maternal influences resulted in more obvious genetic differences and a stronger latitudinal trend.

In retrospect, using region as a proxy for latitude in the principal component analysis and analysis of variance may not have been the most effective method for identifying latitudinal trends. While within each region most of my populations did share a similar latitude, populations within the region I called SI_C were far more latitudinally spread than the other regions (Figure 2.1). This may have hidden any latitudinal trends which were suggested in the ANCOVA.

Maternal effects have been shown to have significant effects on offspring phenotype (Galloway, 2005; Schuler & Orrock, 2012). They have the ability to mask genetic differences

by effecting phenotype expression (Libby & Jund, 1962; Roach & Wulff, 1987; Montalvo, 1994; Galloway, 1995; Schwaegerle *et al.*, 2000; Galloway, 2005; Latzel & Klimesova, 2010; Dong *et al.*, 2017). Despite this background, maternal effects appeared to play a minor role in the elicitation of phenotype for New Zealand populations of *E. guttata*. Instead, I found that genetic diversity governs the phenotypic diversity across the majority of traits with a co-occurring effect of phenotypic plasticity. Subsequently, the loss of maternal effects provided further evidence to support and strengthen the conclusions I made in chapters 2 and 3; there is high genetic diversity and ability to elicit plastic phenotypes as well as evidence for clinal variation associated with a latitudinal gradient in New Zealand populations of *E. guttata*.

5.4 Summary and the potential for control

5.4.1 Summary

Populations of *Erythranthe guttata* were sampled from 35 populations spanning seven geographically distinct regions across New Zealand. Common garden experiments found that both genetic differentiation and phenotypic plasticity were causing the variablity in phenotype observed in the field. This result corresponds to previous studies indicating the most, if not all, genetic variation in native populations of *E. guttata* has been introduced to the invasive ranges of Scotland and New Zealand (van Kleunen & Fischer 2008). *E. guttata* is still used in the New Zealand horticulture which has likely resulted in the continued introduction of genetic variants.

My results concluded that there was evidence for latitudinal trends in phenotypic expression across New Zealand. This conclusion was not surprising considering van Kleunen and Fischer (2008) identified a latitudinal trend in *E. guttata* across North America, Scotland and New Zealand.

An extended search into latitudinal trends in New Zealand plant species provided ambiguous results with a lack of latitudinal trend found for the exotic species *Agrostis capillaris* (Rapson & Wilson, 1992) but the presence of a latitudinal trend was found for the native species *Leptospermum scoparium* (Harris, 2002).

It was important that I collected data for a second year. The second seasons worth of data provided sufficient information to conclude that maternal environment influences offspring

phenotype. The results showed that as maternal effects disspate, the effects of genetic differences became more apparent and a latitudinal trend was more prominent. While phenotypic plasticity was still evident, genetic effects became stronger in controlling phenotypic expression.

5.4.2 Management implications

Riparian regions are regarded as an important source of biodiversity and fulfill a range of ecosystem services (Michalski *et al.*, 2017). They also tend to be highly susceptible to invasion, a consequence of their high resource availability and high disturbance (Miller *et al.*, 2015). Understanding phenotypic diversity, be that via genotypic differentiation or phenotypic plasticity, across heterogenous environments is crucial in controlling invasive species spread. Assumptions about habitat susceptibility to invasiveness and species invasibility often underpin the development of invasive species monitoring programmes. These assumptions provide the rationale for developing management programmes despite a lack of evidence-based research.

Phenotypic diversity does not always equate to genetic diversity. In the case of this study, phenotypic diversity translates to a contribution of both genetic diversity and phenotypic plasticity. Both features play an important role in range expansion and phenotypic plasticity has been found to facilitate genetic differentiation. Essentially, two populations with similar phenotypes could be genetically different and two populations with dissimilar phenotypes could be genetically alike. Without genetic analyses, these possibilities are not easily untangled, however multiple common garden experiments can being to. As such, the results of this study could go a long way to developing evidence-based management programmes for *Erythranthe guttata* in New Zealand. The observed phenotypic variability, consequential of genetic differences and phenotypic plasticity, highlights the importance of varied control and eradication methods.

The observed genetic diversity, likely via multiple introductions and easy propagule (seed and rhizome fragments) spread, suggests that management and eradication of *E. guttata* needs to occur on a national scale as opposed to the current regional scale. While regional councils have made note of *E. guttata* and its invasive properties, the Northland Regional Council is

the only group with a targeted eradication approach. Eradication attempts in Northland alone seem futile considering the easy reintroduction from regions to its south. A national approach to management and eradication of *E.* guttata would attempt to minimise dispersal and reintroduction.

5.4.3 The potential for control

There are two major control techniques for managing *Erythranthe guttata* invasions in New Zealand; hand weeding and herbicide use (Northland Regional Council, 2017; Collins *et al.*, 2018).

Hand weeding:

Hand weeding of *Erythranthe guttata*, while effective, is laborious and expensive (Collins *et al.*, 2018). The regenerative characteristics of *E. guttata* make it suitably adapted to stochastic and variable conditions (Truscott *et al.*, 2006). It is difficult to remove entire plants without leaving behind rhizome fragments and therefore requires regular maintenance. Despite this, it is the most targeted eradication approach for removing *Erythranthe guttata* and can be hugely successful at removing small infestations (Collins *et al.*, 2018).

Herbicides:

The ability to control *Erythranthe guttata* with herbicides is debatable. Careful use of herbicides must be implemented due to the close proximity with freshwater systems. Improper use could result in downstream effects to the biodiversity in the freshwater system. For example, there is risk of harm to the native fish and invertebrate species not just in the immediate area, but further afield.

The rhizomatous nature of growth means that killing an entire plant/population with one herbicide treatment is unlikely. Instead, a follow up treatment twice yearly is recommended to prevent the establishment and growth of seedlings. A lag period of approximately two months between application and senescence has been observed by Collins *et al.* (2018). They suggested targeting spraying to early in the growing season so as to maximally affect early seedling stages.

5.5 Future work

As it stands, there are several avenues for future research. Invasion biology is still in its infancy and only in the last decade has it garnered significant attention. Further research into New Zealand populations of *Erythranthe guttata* could follow multiple avenues. The most depauperate area is in the molecular research. In particular, the understanding of genetic structure. Several questions remain unanswered including:

- Does *E. guttata* exhibit pre-adaption in its native range that facilitates invasion success or does it undergo post-invasion adaptation? What is the relative importance of these two methods for invasive expansion?
- Do traits or trait combinations of *E. guttata* predict its invasion success? If yes, are these traits or trait combinations heritable?
- Does *E. guttata* possess key traits that mediate invasive behaviour? If yes, are these genes unique to *E. guttata* or are they shared by multiple species?
- Is the prolific exapansion of *E. guttata* driven by genetically-derived traits, environmental features such as disturbance, or the interaction of the two?
- Do maternal effects enhance performance of *E. guttata* and facilitate invasion success?

Following the conclusions of chapter 3, further research into latitudinal trends of both native and exotic species is essential. Moving away from *E. guttata* specifically, the field of latitudinal trends in New Zealand plant species is severly lacking. Further research could identify the prevalence of latitudinal trends in the New Zealand flora.

Answering these questions and further investigation into latitudinal trends would increase our understanding around the rates of biodiversity change and biodiversity loss in New Zealand. Moreover, understanding population genetic structure in New Zealand populations of *E. guttata* is pivotal for predicting further spread and the likelihood of evolving resistance to herbicide use. It could provide local councils with the knowledge to develop legislation around land and resource management practices which would move New Zealand towards a future less threatened by biodiversity loss and prevent ecosystem domination by exotic pest species. It should be remembered that biotic change is a natural evolutionary process (Waters

& Grosser, 2016), however, in an environment increasingly affected by anthropogenic global change, understanding these processes is indispensable for mitigating the detrimental effects of invasions and fostering the survival of native biodiversity and the richness of New Zealand's native species.

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Appendix

Appendix A.

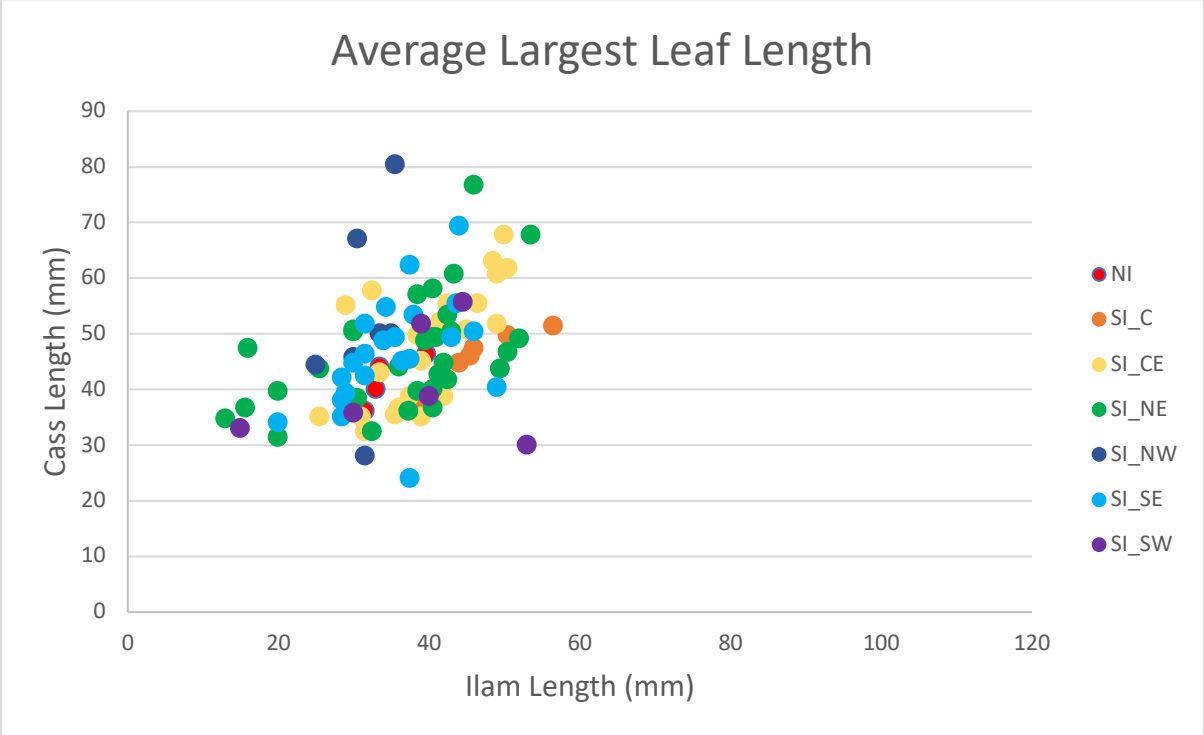
Analysis of variance for differences in 13 first year performance measures among populations at both common garden locations.

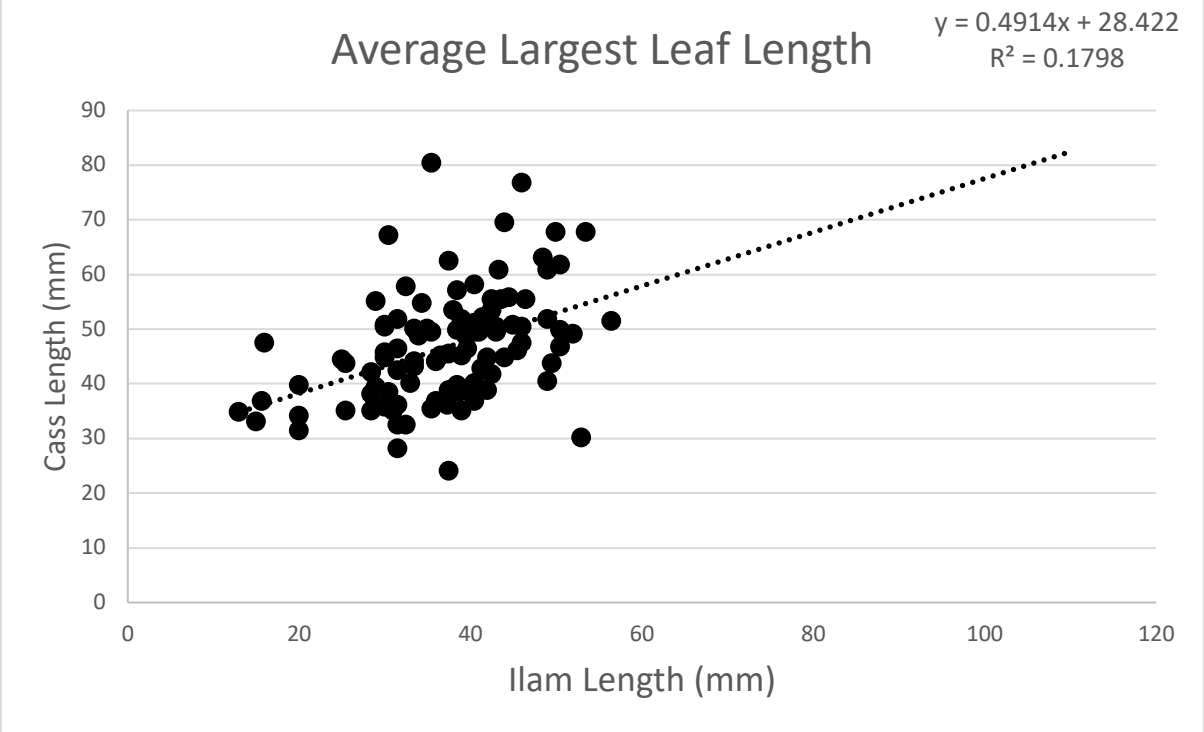
**Significant ($p < 0.05$).*

Performance Measure	Ilam			Cass		
	F value	df	p value	F value	df	p value
Log Above Ground Dry Weight	2.4	137	**0***	1.54	79	0.09
Log Average Largest Leaf Length	2.83	137	**0***	0.83	79	0.67
Log Average Largest Leaf Width	2.75	137	**0***	1.02	79	0.45
Log Longest Horizontal Shoot	2.79	136	**0***	2.6	78	**0***
Longest Vertical Shoot	2.29	77	**0.01***	0.96	33	0.52
Log Internode Length	3.02	137	**0***	1.45	79	0.13
Anthocyanin Score	3.57	137	**0***	2.86	79	**0***
Jdate First Bud	1.25	19	0.26	2.54	30	**0.01***
Jdate Max Bud	0.65	44	0.85	1.48	30	0.17
Max Bud Number	1.52	140	0.08	5.3	79	**0***
Largest Flower Height	0.84	28	0.65	1.31	24	0.27
Largest Flower Depth	0.82	28	0.67	1.35	24	0.25
Largest Flower Width	0.89	28	0.6	1.27	24	0.3

Appendix B.

Example linear regressions: the average largest leaf length for plants grown at Ilam and plants grown at Cass. The first linear regression identifies individuals by region, the second linear regression observes the coefficient of determination for all individuals, regardless of region, and the equation of the trendline.





Appendix C.

The code I used to produce the linear mixed-effects models.

```
subdata <- data[, c("Performance Measure", "Garden.Location", "Region", "Population")]
library(nlme)
mod <- lme((Performance Measure) ~ 0+Garden.Location, data=na.omit(subdata),
        random=list(Region=pdDiag(~0+Garden.Location),
                Population=pdDiag(~0+Garden.Location)),
        weights=varIdent(form=~1|Garden.Location),
        control=nlmeControl(maxIter=1000))
summary(mod)
intervals(mod)
```

Appendix D.

Linear mixed effects model for the 13 first year performance measures at both garden locations. Includes lower and upper confidence intervals (CI).

**Significant difference between the performance measure at Ilam and at Cass (p < 0.05).*

Response Variable		Ilam			Cass		
		Estimate	CI lower	CI upper	Estimate	CI lower	CI upper
Log Above Ground Dry Weight	Mean	1.68	1.19	2.17	2.29	2.09	2.5
	Region StdDev	0.58	0.27	1.21	0	0	inf
	Population StdDev	0.57	0.38	0.85	0.28	0.1	0.78
	Clone StdDev	1.06	0.96	1.16	0.85	0.71	1.02
Log Average Largest Leaf Length*	Mean	3.64	3.54	3.74	3.82	3.78	3.86
	Region StdDev	0.1			0		
	Population StdDev	0.17			0		
	Clone StdDev	0.3			0.22		
Log Average Largest Leaf Width*	Mean	3.38	3.25	3.51	3.64	3.59	3.69
	Region StdDev	0.13			0		
	Population StdDev	0.2			0.02		
	Clone StdDev	0.29			0.24		
Log Longest Horizontal Shoot*	Mean	5.13	4.91	5.35	4.81	4.61	5
	Region StdDev	0.24	0.11	0.53	0.18	0.05	0.71
	Population StdDev	0.29	0.2	0.42	0.2	0.09	0.44
	Clone StdDev	0.49	0.44	0.54	0.43	0.36	0.52
Longest Vertical Shoot	Mean	155.93	131.56	180.29	134.2	109.46	158.95
	Region StdDev	7.51	0	inf	10.6	0.05	2491.08
	Population StdDev	44.14	21.18	91.91	18.1	1.43	228.87
	Clone StdDev	94.42	82.02	108.7	74.51	57.26	96.95
Log Internode Length	Mean	3.4	3.19	3.62	3.51	3.39	3.62
	Region StdDev	0.24	0.1	0.55	0	0	inf
	Population StdDev	0.3	0.21	0.44	0.14	0.04	0.51
	Clone StdDev	0.51	0.47	0.56	0.51	0.42	0.61
Anthocyanin Score	Mean	2.08	1.7	2.46	1.76	1.58	1.94
	Region StdDev	0.44	0.21	0.91	0	0	inf
	Population StdDev	0.54	0.4	0.73	0.33	0.2	0.55
	Clone StdDev	0.6	0.54	0.65	0.54	0.45	0.65
Jdate First Bud	Mean	12.73	8.44	17.01	19.08	13.63	24.53
	Region StdDev	3.93			0		
	Population StdDev	6.34			8.1		
	Clone StdDev	17.65			20.88		
Jdate Max Bud	Mean	14.62	9.67	19.54	23.12	15.7	30.54
	Region StdDev	4.46			0		
	Population StdDev	7.54			13.01		
	Clone StdDev	19.98			24		
	Mean	5.48	4.44	6.52	5.76	3.98	7.5

Max Bud Number	Region StdDev	0.64	0.06	6.72	1.32	0.26	6.71
	Population StdDev	1.87	1.08	3.23	1.9	0.69	5.24
	Clone StdDev	5.36	4.89	5.87	5.29	4.43	6.33
Largest Flower Height	Mean	26.59	25.05	28.13	25.25	23.14	27.37
	Region StdDev	0.76	0.04	16.37	0	0	inf
	Population StdDev	0	0	inf	2.24	0.75	6.7
	Clone StdDev	5.59	4.68	6.69	5.1	3.71	7
Largest Flower Depth	Mean	39.06	37.8	40.32	40.89	37.7	44.09
	Region StdDev	0	0	inf	2.23	0.44	11.34
	Population StdDev	0.7	0	305.92	1.77	0.19	16.4
	Clone StdDev	4.9	3.97	6.05	6.19	4.4	8.7
Largest Flower Width	Mean	29.63	28.19	31.07	31.4	28.63	34.16
	Region StdDev	0			0.54		
	Population StdDev	1.6			2.84		
	Clone StdDev	5.09			6.56		

Appendix E.

Exponential linear mixed effects model for the 13 first year performance measures at both Ilam and Cass common gardens. Includes lower and upper confidence intervals (CI).

**Significant difference between the performance measure at Ilam and at Cass ($p < 0.05$).*

Response Variable		Ilam			Cass		
		Estimate	CI lower	CI upper	Estimate	CI lower	CI upper
Above Ground Dry Weight	Mean	9.64	6.86	12.41	13.64	11.41	15.87
	Region StdDev	3.02	1.25	7.27	0.33	0	inf
	Population StdDev	3.31	1.94	5.67	2.34	0.52	11.4
	Clone StdDev	9.07	8.26	9.96	9.79	8.17	11.74
Average Largest Leaf Length *	Mean	40.32	36.59	44.04	46.71	44.67	48.75
	Region StdDev	3.24			0		
	Population StdDev	6.95			0		
	Clone StdDev	11.37			10.35		
Average Largest Leaf Width *	Mean	31.29	27.86	34.73	39.15	37.26	41.05
	Region StdDev	3.49			0		
	Population StdDev	5.6			1.49		
	Individual StdDev	8.54			9.02		
Longest Horizontal Shoot *	Mean	196.74	162.04	231.44	137.36	116.4	158.31
	Region StdDev	37.82	16.49	86.73	16.73	2.72	102.99
	Population StdDev	49.21	33.73	71.8	25.22	11.77	54.03
	Clone StdDev	85.43	77.71	93.92	53.26	44.31	64.03
Internode Length	Mean	34.75	28.4	41.1	37.23	33.64	40.82
	Region StdDev	7.24			0		
	Population StdDev	8.52			4.81		
	Clone StdDev	13.45			14.68		

Appendix F.

Two-way analysis of covariance of garden location and population on all 13 first year performance traits observed to vary in the field. Degrees of freedom for the residual is: 297 for log above ground dry weight, log average largest leaf length and width, internode length and anthocyanin score; 292 for log longest horizontal shoot; 133 for vertical shoot; 87 for jdate first bud; 86 for jdate max bud; 310 for max bud number; 61 for largest flower height, depth and width.

**Significant ($p < 0.05$)*

Performance Measure		**Source of Variation**		
		Garden Location	Population	GL*Population
Log Above Ground Dry Weight	F-statistic	22.48	5.35	1.20
	p-value	**<0***	**<0***	0.26
	% Variance	4	35	5
Log Average Largest Leaf Length	F-statistic	30.86	4.48	0.79
	p-value	**<0***	**<0***	0.72
	% Variance	6	31	3
Log Average Largest Leaf Width	F-statistic	63.41	6.08	0.85
	p-value	**<0***	**<0***	0.65
	% Variance	11	35	3
Log Longest Horizontal Shoot	F-statistic	30.23	6.06	1.17
	p-value	**<0***	**<0***	0.28
	% Variance	5	37	4
Longest Vertical Shoot	F-statistic	1.84	1.91	1.21
	p-value	0.18	**≤0***	0.26
	% Variance	1	27	10
Log Internode Length	F-statistic	1.99	4.93	1.35
	p-value	0.16	**<0***	0.15
	% Variance	0	34	5
Anthocyanin Score	F-statistic	7.79	12.21	1.53
	p-value	**0.01***	**<0***	0.07
	% Variance	1	55	4
Jdate First Bud	F-statistic	3.56	1.90	2.14
	p-value	0.06	**0.01***	**0.02***
	% Variance	2	29	18
Jdate Max Bud	F-statistic	12.12	1.24	1.21
	p-value	**≤0***	0.23	0.28
	% Variance	8	22	12
Max Bud Number	F-statistic	1.05	2.20	1.57
	p-value	0.31	**≤0***	0.06
	% Variance	0	18	8
Largest Flower Height	F-statistic	0.91	1.16	1.12
	p-value	0.34	0.31	0.36

	% Variance	0	9	5
Largest Flower Depth	F-statistic	4.10	1.17	1.09
	p-value	**0.05***	**0.30***	0.38
	% Variance	4	28	13
Largest Flower Width	F-statistic	2.78	1.19	1.11
	p-value	0.10	0.28	0.37
	% Variance	3	28	13

Appendix G.

Three-way analysis of covariance for location, latitude and region on all 13 first year performance traits. Degrees of freedom for the residual is: 329 for log above ground dry weight, log average largest leaf length, log average largest leaf width, log internode length, anthocyanin score; 324 for log longest horizontal shoot; 158 for vertical shoot; 108 for jdate first bud; 107 for jdate max bud; 342 for max bud number; 80 for largest flower height, depth and width.

**Significant ($p < 0.05$)*

Source of Variation	Log Above Ground Dry Weight			Log Average Largest Leaf Length			Log Average Largest Leaf Width			Log Longest Horizontal Shoot			Longest Vertical Shoot			Log Internode Length			Anthocyanin Score		
	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance
Garden Location	19.99	**≤0***	4	50.72	**<0***	11	50.72	**<0***	11	25.65	**<0***	5	1.69	0.19	10	1.74	0.19	0	6.08	**0.01***	1
Latitude	1.21	0.27	0	0.25	0.61	0	0.25	0.61	0	1.11	0.26	0	0.10	0.75	0	4.35	**0.04***	1	11.18	**≤0***	2
Region	14.48	**<0***	19	11.44	**<0***	15	11.44	**<0***	15	15.04	**<0***	19	1.68	0.13	5	11.82	**<0***	16	33.16	**<0***	34
GL*Latitude	≤0	0.94	0	1.43	0.23	0	1.43	0.23	0	2.53	0.11	1	0.50	0.48	0	6.20	**0.01***	1	0.18	0.67	0
GL*Region	0.86	0.52	1	0.51	0.80	1	0.51	0.80	1	1.33	0.24	2	2.07	0.06	6	1.04	0.40	1	1.14	0.34	1
Latitude*Region	2.65	**0.02***	3	2.41	**0.04***	3	2.41	**0.04***	3	2.31	**0.04***	2	1.91	0.10	5	1.55	0.17	2	5.49	**<0***	5
GL*Latitude*Region	3.85	**0.01***	2	0.71	0.54	0	0.71	0.54	0	1.68	0.17	1	1.09	0.36	2	1.25	0.29	1	0.87	2.01	1

Appendix G. continued

Source of Variation	Jdate First Bud			Jdate Max Bud			Max Bud Number			Largest Flower Height			Largest Flower Depth			Largest Flower Width		
	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance	F-statistic	p-value	% Variance
Garden Location	3.50	0.06	2	12.81	**≤0***	8	0.97	0.33	0	0.89	0.35	1	4.16	**0.04***	4	2.88	0.09	3
Latitude	2.60	0.11	2	1.12	0.29	1	0.24	0.63	0	0.66	0.42	1	0.55	0.46	0	0.56	0.45	0
Region	2.74	**0.02***	10	0.88	0.51	3	2.30	**0.03***	4	1.07	0.39	6	0.99	0.44	5	1.02	0.42	5
GL*Latitude	5.61	**0.02***	3	7.54	**0.01***	5	1.59	0.21	0	0.19	0.67	0	0.51	0.48	0	1.37	0.25	1
GL*Region	1.20	0.31	4	1.32	0.25	5	2.65	**0.02***	4	1.19	0.32	6	1.84	0.11	8	2.12	0.07	9
Latitude*Region	2.20	0.06	6	1.09	0.37	3	1.81	0.11	2	1.51	0.20	7	1.42	0.22	6	1.98	0.09	9
GL*Latitude*Region	8.25	**≤0***	10	493.02	5.47	7	0.52	0.67	0	1.32	0.27	3	1.89	0.16	3	0.92	0.40	2

Appendix H.

Two-way analysis of covariance of region and latitude on all 15 second year performance measures. Degrees of freedom for the residual is: 300 for log largest leaf surface area and log max flower number; 298 for leaf length, leaf width and leaf shape; 299 for the longest horizontal shoot, longest vertical shoot and internode length; 312 for the jdate first bud; 271 for the jdate first flower; 263 for the jdate max flower; 250 for the log petiole length; 248 for the flower height, depth and width.

**Significant ($p < 0.05$).*

Performance Measure		**Source of Variation**		
		Region	Latitude	Region*Latitude
Log Largest Leaf Surface Area	F-statistic	13.31	4.62	4.78
	p-value	**<0***	**0.03***	**≤0***
	% Var.	20	1	6
Leaf Length	F-statistic	11.27	6.88	5.98
	p-value	**<0***	**0.01***	**<0***
	% Var.	17	2	7
Leaf Width	F-statistic	13.94	2.26	4.68
	p-value	**<0***	0.13	**≤0***
	% Var.	60	2	17
Leaf Shape	F-statistic	9.23	5.60	2.82
	p-value	**<0***	**0.02***	**0.02***
	% Var.	15	2	4
Longest Horizontal Shoot	F-statistic	16.54	9.58	4.14
	p-value	**<0***	**≤0***	**≤0***
	% Var.	23	2	5
Longest Vertical Shoot	F-statistic	10.45	11.43	2.51
	p-value	**<0***	**≤0***	**0.03***
	% Var.	16	3	3
Internode Length	F-statistic	4.84	5.32	2.45
	p-value	**<0***	**0.02***	**0.03***
	% Var.	8	2	4
Jdate First Bud	F-statistic	10.51	20.86	1.85
	p-value	**<0***	**<0***	0.10
	% Var.	16	5	2
Jdate First Flower	F-statistic	12.82	15.43	4.96
	p-value	**<0***	**≤0***	**≤0***
	% Var.	20	4	6

Appendix H. continued

		Region	Latitude	Region*Latitude
Jdate Max Flower	F-statistic	3.24	4.56	1.60
	p-value	**≤0***	**0.03***	0.16
	% Var.	7	2	3
Log Max Flower Number	F-statistic	3.91	11.40	4.35
	p-value	**≤0***	**≤0***	**≤0***
	% Var.	7	4	7
Log Petiole Length	F-statistic	23.91	5.85	6.91
	p-value	**<0***	**0.02***	**<0***
	% Var.	33	1	8
Largest Flower Height	F-statistic	8.74	5.69	2.07
	p-value	**<0***	**0.02***	0.07
	% Var.	17	2	3
Largest Flower Depth	F-statistic	12.18	0.11	0.47
	p-value	<0*	0.74	0.80
	% Var.	23	0	1
Largest Flower Width	F-statistic	16.03	0.07	2.58
	p-value	**<0***	0.79	**0.03***
	% Var.	27	0	4